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SECONDARY ANEMIA OF INFANTS

A STUDY OF SO-CALLED INFANTILE SPLENIC ANEMIA OR ANEMIA INFANTUM PSEUDOLEUKEMICA

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It is evident from a study of pediatric and hematologic literature that great confusion exists concerning the nature and significance of the various types of anemia occurring in infancy, particularly in regard to that symptom complex spoken of as von Jaksch's anemia. This confusion is due to the attempt to apply the criteria used for adults to the interpretation of the blood picture in infants, and also to the failure to appreciate the peculiarities of the response of the infantile hematopoietic system to infection and anemia. A lymphocytic reaction is frequently observed in infants with anemia and has been confused with leukemia. Enlargement of the spleen, prematurity, faulty feeding, nutritional disorders, rickets, and other conditions, introduce additional factors which complicate the study of blood disorders in infancy. These various factors must not be considered singly, but in relation to each other, if we would appreciate fully the peculiarities of the blood picture observed in infants. This report is based on a study of infants with anemia. The attempt will be made to show that the anemias so frequently encountered in infants with rickets, nutritional disorders, and other conditions associated with enlargement of the spleen, liver or

lymph nodes, and with quantitative and qualitative changes in the blood picture (such as, anemia, leucocytosis, erythroblastosis, lymphocytosis, myelocytosis) are merely secondary anemias with varying predominant types of reaction.

Although some studies of infantile anemias had been made by Gretsell¹ in 1866 and by Somma² in 1884, it was not until the appearance of papers by von Jaksch³ in 1899 and 1890 that attention was directed to a peculiar type of anemia often seen in infancy. Von Jaksch described four cases in his two papers which showed, in addition to a diminution in the red blood cells and hemoglobin, some poikilocytosis, a leucocytosis and enlargement of the liver, spleen and lymph nodes. Rickets was present in at least two of these cases. No mention was made of qualitative changes in the blood picture other than increase of eosinophiles. Von Jaksch believed that the condition encountered in these patients was a clinical entity; and that it was to be differentiated from leukemia because the liver was not so large, the anemia was more severe, rickets was usually present, and the malady in itself was not fatal. He suggested the name "anemia infantum pseudoleukemica." At about the same time Di Lorenzo⁴ described some cases

showing essentially similar features and expressed the opinion that they were due to an infectious agent. He used the term "anemia splenica infettiva dei bambini." Hayem,⁶ in 1889, also presented a case of anemia in an infant and called attention for the first time to the presence of nucleated red blood cells. Luzet⁷ in presenting a series of similar cases two years later, emphasized not only the leucocytosis and splenomegaly, but also the presence of nucleated red blood cells. He excluded from this group any patient with rickets or syphilis. The early papers, although not establishing the status of these cases very clearly, served to stimulate interest in them. A number of contributions on the subject followed, and much discussion arose as to whether the cases described were splenic anemia or leukemia, whether they were dependent upon rickets, or were infectious in origin. They were often referred to as von Jaksch-Hayem-Luzet anemia. In general, the following opinions were set forth:

1. Splenic anemia of infants was a secondary anemia (Fischl,¹ Ostrowski,⁸ Naegeli,⁹ Stengel,¹⁰ Ashby,¹¹ Wentworth,¹² Flesch,¹³ Lichtenstein¹⁴).

2. It represented an aleukemic stage of true leukemia (Luzet⁷).

3. It represented a transition between pernicious anemia and leukemia (Engel¹⁵).

4. It stood midway between simple anemia and leukemia (Hutchison¹⁶).

5. It was a disease *sui generis*, a primary anemia (von Jaksch,⁵ Alt and Weiss,¹⁷ Melland,¹⁸ Fowler¹⁹).

6. It represented a myeloid leukemia of infancy (Lehrndorf²⁰).

7. It was a splenomedullary pseudo-leukemia (Pappenheim²¹).

8. It was a primary disorder of the bone-marrow due to rickets (Marfan,²² Aschenheim and Benjamin²³).

Although the significance of the findings in these cases was so little understood, the criteria for diagnosis became more numerous and more clearly defined as more cases were described. Gradually there developed a definite clinical picture usually spoken of as "anemia infantum pseudoleukemica" or "splenic anemia of infants." Infants chiefly from six to twenty months of age were affected. Frequently no other disease was demonstrable, rickets was often, though not always, present, and occasionally prematurity and various infections as syphilis, tuberculosis, malaria, leishmaniasis, were found. A waxy or lemon yellow color of the skin was commonly observed. The spleen was always enlarged, but the enlargement might be either slight or of severe grade. The liver was often a little enlarged, but sometimes was of normal size. There was slight general enlargement of the lymph nodes in some cases. Anemia either slight, moderate, or severe was always present with a color index of one or less than one and with poikilocytosis, anisocytosis and polychromasia. The total white blood cell count was sometimes normal, but there was often a leucocytosis, occasionally very severe. A relative lymphocytosis was common; and immature myeloid cells, pathological lymphoid forms, and nucleated red blood cells

were frequently found, sometimes in great numbers. The mortality was high, due not to the condition *per se*, but rather to secondary infection. It is interesting that this clinical picture, which is so much broader in its scope than the one originally described by von Jaksch, is still frequently referred to as "von Jaksch's anemia."

All the various manifestations encountered in these cases excited discussion, and attempts were made to determine the relation of each to the entire symptom complex. The frequency of rickets as an associated condition was noted early, and mentioned in nearly every report on the subject. Hutchison,¹⁶ in 1904, observed that the rickets was usually not of severe degree, that the cranial bones were frequently affected and showed bosses in the frontal and parietal regions. Rickets was suggested by some as the etiological factor. Aschenheim and Benjamin²³ collected seventy cases from the literature and they considered the relation of rickets to "anemia infantum pseudoleukemica" the same as that of syphilis to tabo-paresis. They considered the disease a degenerative process of the bone-marrow and coined the term "Rachitische Megalosplenie" to emphasize the associated rickets. Aschenheim,²⁴ in 1912, studied the blood in rickets and attempted to differentiate rickets with mild anemia from rickets with severe anemia. He found that the severity of the rickets was not parallel with the severity of the anemia. Marfan,²² also, held that the condition was a disorder of the bone-marrow due to rickets.

The variable number of leucocytes found in different cases and in the same case at different times excited comment. Many authors (Ashby,¹¹ Wentworth,¹² Aschenheim and Benjamin,²³ Lichtenstein¹⁴ and others) insisted that leucocytosis is not essential to the blood picture, that the white blood cells might be diminished, normal, or increased in number, and that very high counts are exceptional.

A relative and absolute increase in lymphocytes has been mentioned repeatedly (Fischl,¹ Geissler and Japha,²⁵ Fowler,¹⁹ Weil and Clerc,²⁶ Riviere,²⁷ Flesch and Schosberger,²⁸ Kleinschmidt,²⁹ Hunter,³⁰ Hutchison,¹⁶ Aschenheim and Benjamin,²³ Ashby,¹¹ Ostrowsky,⁸ Stettner,³¹ Lichtenstein,¹⁴ Hallez³²). In the case presented by Geissler and Japha, 97 per cent of the white blood cells were lymphocytes, but unfortunately not enough data are available to rule out the possibility of leukemia in this instance. Hutchison reported a case with 79 per cent lymphocytes, Stettner one with 68 per cent, and Lichtenstein one with 81 per cent. This lymphocytosis was often associated with enlargement of the lymph nodes. Both normal and pathological lymphocytes were present. The significance of the lymphoid reactions occasioned some discussion, but they were attributed by most observers merely to the unusual lymphoid activity in infancy.

The pronounced differences in the degree of enlargement of the spleen in these cases has also been given some attention. The variability and consequent lack of importance of the enlargement of the spleen were emphasized by Fischl,¹ Wentworth,¹² Hutchison,¹⁶ Aschenheim,²⁴ and others. Carpenter³³ stated, "The anemia may be extreme and yet there be no enlargement of the spleen, and on the other hand quite an

appreciable enlargement of the spleen is frequently associated with a very mild form of anemia."

The frequency of infection so often associated with this symptom complex, and the rôle of infection in the production of severe anemia in infancy has been emphasized by Stettner²¹ and others. Particularly in Italy the importance of malaria and leishmaniasis has been pointed out.

In addition to the anemias described above under the terms splenic anemia of infants or anemia infantum pseudoleukemica, others have been described as a separate group; "chlorotic anemia of infants," "alimentary anemia" (Kleinschmidt,²² Swartz and Rosenthal,²³ Lichtenstein²⁴). In these cases the blood picture varied from merely a reduction of hemoglobin to one approaching the type presented above. The anemia was attributed to a deficient supply of iron due to a prolonged milk diet or to underfeeding. Likewise, the frequent occurrence of anemia in premature infants has been noted often, and attributed to a deficient store of iron (Bunge²⁵) and to a failure of the hematopoietic system to develop (Lichtenstein²⁴). The anemias of these infants presented a blood picture of the simple chlorotic type or one resembling rather closely that referred to as "anemia infantum pseudoleukemica."

In this study only cases have been presented in which syphilis, tuberculosis, all known specific causes for enlargement of the spleen, liver and lymph glands, and all primary diseases of these organs and the bone-marrow, were ruled out, as far as possible, by clinical procedures. Each case was, therefore, an example of so-called "infantile splenic anemia," or "anemia infantum pseudoleukemica."*

CASE 1.—C. R., a white infant, aged nine months, was admitted to The Harriet Lane Home March 18, 1920, because of failure to gain and anemia.

The patient was born prematurely at the seventh month. Breast feeding was attempted but was unsuccessful, and he was artificially fed on Mellin's food and condensed milk up to the time of admission to the hospital. He received in addition cod-liver oil and orange juice.

Examination on admission showed a poorly nourished infant weighing 12 pounds. The skin and mucous membranes were very pale. The head was large in proportion to the body, square, with prominent frontal bosses, between which there was a deep depression. The sutures were closed, but the anterior fontanelle was widely open and slightly depressed. There was slight cranio-tabes. The eyeballs were rotated downwards, exposing the sclera above the cornea. The lungs were clear. There was no enlargement of the epiphyses of the long bones, nor any deformities of the extremities, thorax or spine. The anterior and posterior cervical glands were palpable, but there was no general lymph-glandular enlargement. The spleen was enlarged,

* The abbreviations for white blood cells in the blood charts are as follows:

P. M. N.—polymorphonuclear neutrophile.

P. M. E.—polymorphonuclear eosinophile.

P. M. B.—polymorphonuclear basophile.

S. L.—normal small lymphocyte.

L. L.—normal large lymphocyte.

P. L.—pathological lymphoid forms.

L. M. }—cells of the large mononuclear-transitional cell group—
Trans. } oxydase mononuclears.

The hemoglobin is expressed in percentage, as estimated by the Sahli apparatus.

filling the entire left flank, the lower edge extending to the anterior superior spine. The edge was firm and smooth. The liver edge was just felt at the right costal margin.

The Pirquet and Wassermann tests were negative. X-rays of the head, chest and extremities showed mild rickets. The blood picture showed moderate reduction in red blood cells, a severe reduction of hemoglobin, slight anisocytosis and poikilocytosis, rather pronounced basophilia and a few nucleated forms; and a leucocytosis with an essentially normal differential formula (see Table I).

During his stay in the hospital the infant developed otitis media and acute bronchitis with elevations of the temperature to 102° F. Treatment consisted in a diet of milk, barley water and sugar mixture, cereals, broth, orange juice, and cod-liver oil (one drachm three times a day). He improved somewhat and reached a weight of 13½ pounds on April 21, 1920, when he was discharged. He has not been seen since that time.

This case, of a premature infant, artificially fed, with mild rickets and severe infections, showed, with a marked enlargement of the spleen and slight enlargement of the liver, merely a simple secondary anemia. The findings in this patient are to be compared with those in Case 2 in which there was slight enlargement of the spleen, and with those in Case 3 in which splenomegaly was not present. In both of these cases the blood picture was essentially the same as in this patient, differing from it only in the degree of anemia present and the percentage of lymphocytes found.

CASE 2.—E. S., a white infant, aged 11 months, was admitted to The Harriet Lane Home May 19, 1920, because of failure to gain weight.

Birth was premature at the seventh month; the birth weight was 2½ pounds. The patient did not gain properly, although various milk preparations were tried and at times a wet nurse was secured. For two months the patient had had breath-holding spells. There had been no vomiting or diarrhea.

Examination on admission showed a small, undernourished infant, weighing 8 pounds. The skin and mucous membranes were pale. The head was short anteroposteriorly with high forehead and full fontanelle. Cranio-tabes was present. The epiphyses of the long bones were slightly enlarged and X-rays of these showed rickets. The abdomen was distended. There was no enlargement of the lymph nodes. The spleen was felt one finger's breadth below the costal margin. The liver was not felt.

The Pirquet and Wassermann tests were negative. The blood in this case showed a moderate reduction in red blood cells and hemoglobin, some anisocytosis and poikilocytosis, slight diffuse basophilia; and a slight leucocytosis at one time with a moderate lymphoid reaction (see Table II).

The patient has remained in the hospital since admission. She has gained slowly in weight (at present she weighs 13 pounds). Cereals and meat were added to the milk diet and she was given, in addition, cod-liver oil and reduced iron. There has been slight improvement in her general condition.

This case is that of a premature child, badly fed and with mild rickets, who showed, with a slightly enlarged spleen, a blood picture essentially that of a simple secondary anemia. This case is to be compared with Case 1 having a large spleen, and with Case 3 showing no enlargement of the spleen, both of which presented the same type of blood picture.

CASE 3.—G. E., a colored infant, aged eight months, was admitted to The Harriet Lane Home May 12, 1920, because of fever.

Birth was premature at the seventh month. The patient was breast fed for three months and then weaned to a whole milk mixture supplemented by orange juice, but no other food. For two weeks there

had been fever and difficulty in breathing. Examination on admission showed a fairly well-nourished infant weighing 12 pounds. The skin and mucous membranes were strikingly pale. There was enlargement of the epiphyses and costochondral junctions, but no deformities. A systolic murmur was heard over the heart, and scattered patchy consolidations of the lungs were made out. There was slight general enlargement of the lymph nodes. The liver and spleen were not felt.

The Pirquet and Wassermann tests were negative. X-rays of the extremities showed rickets. The blood in this case showed a moderately severe reduction in the number of red blood cells, a severe reduction in hemoglobin, moderate anisocytosis and poikilocytosis, slight basophilia, a few nucleated forms, and a moderately severe leucocytosis, showing the presence of practically no immature forms but with a wide variation in the relative percentage of myeloid and lymphoid cells between counts done ten days apart (see Table III).

The patient reacted poorly to the pulmonary infection and died May 24, 1920. *Autopsy findings:* Rickets, confluent pneumonia, fatty changes in heart and liver.

This patient born prematurely and then artificially fed and having severe rickets and an acute infection, with slight general glandular enlargement but no enlargement of the spleen or liver, showed a blood picture essentially that of a simple secondary anemia with leucocytosis. This case is to be compared with Case 1 in which a premature infant with mild rickets showed, in the presence of marked enlargement of the spleen, about the same blood picture. The reaction of the blood is of interest in connection with the question, "Are the blood changes seen in so-called von Jaksch's anemia merely an infantile response to demands for increased hematopoietic activity?" Here there is an anemia and a call for leucocytes, yet the blood picture is not that commonly spoken of as von Jaksch's anemia, but only that of a simple secondary anemia with leucocytosis.

CASE 4.—D. B., a white infant, aged nine months, was admitted to The Harriet Lane Home August 23, 1919, because of anemia.

The patient was a premature infant, born at the sixth month and was said to have weighed only two pounds at birth. He was never breast fed. Condensed milk feedings were given for four months, and following this a whole milk mixture with orange juice. He was able to sit up and had gained fairly well in weight, but for several months the mother had noticed a pale yellow color of the skin.

Examination on admission showed a fairly well-nourished white boy, weighing 13½ pounds. The skin and mucous membranes were pale with a lemon yellow tint. There was, however, no jaundice. The head was large with prominent frontal bosses and flattened posteriorly. The anterior fontanelle was almost closed. The thorax was of normal shape. There was a systolic murmur heard over the heart. There was slight epiphyseal enlargement of the long bones but no deformities. The lymph glands were not enlarged. The spleen was felt 2 cm. below the left costal margin, definitely palpable but not much enlarged. The liver was not palpable.

The Pirquet and Wassermann tests were negative. The blood in this case showed a moderately severe reduction in red blood cells and hemoglobin, a minimal amount of anisocytosis and poikilocytosis, a slight grade of diffuse basophilia, with no nucleated forms; and a moderate leucocytosis, at one time a high lymphocyte percentage, and also the presence of a few immature myeloid cells (see Chart IV).

The patient was given a diet consisting of whole milk mixture and cereals. He was taken to another hospital August 26, 1919, and there transfused with 75 c.c. of whole blood. He was readmitted to The Harriet Lane Home November 25, 1919, having developed breath-holding spells. Examination revealed no new features, except that the spleen was not palpable. The anemia persisted on a mixed diet

with meat and broth. With the administration of ferrum reductum (gr. II, t. i. d.) he improved and was discharged December 14, 1919. When last examined April 15, 1920, he showed marked improvement.

This case of a premature infant, artificially fed, and having very mild rickets showed, with a slight enlargement of the spleen and no enlargement of the liver, a blood picture resembling very closely that seen in Case 5, an infant born at term in which there was no rickets but a moderate enlargement of both the spleen and liver.

CASE 5.—J. M., a white boy, aged two years and ten months, was admitted to The Harriet Lane Home September 9, 1919, because of anemia and fever.

The patient was born at term, and was breast fed for two months. After weaning he was given a cow's milk mixture with the gradual addition of other foods so that prior to admission he was taking a general mixed diet including green vegetables, meat and eggs.

Examination on admission showed a well-nourished boy, well developed and active. There was marked pallor of the skin and mucous membranes. The head was somewhat large in proportion to the body and the frontal bosses were prominent. There were no other evidences of rickets. A systolic murmur was heard over the precordium. The cervical, axillary and inguinal lymph glands were moderately enlarged. The spleen edge was felt three fingers' breadth below the left costal margin. There was a bilateral otitis media and myringotomy was performed. Examination of the naso-pharynx showed a mass of adenoid tissue.

The Pirquet and Wassermann tests were negative. The temperature was slightly elevated (100° F.), due to the otitis media. The blood showed a slight diminution of the red blood cells, a more severe diminution in hemoglobin, a minimal amount of anisocytosis and poikilocytosis, practically no basophilia, no nucleated forms; and a slight leucocytosis showing a moderate lymphoid reaction with, at times, the presence of many immature forms, and an occasional immature myeloid cell (see Table V).

Treatment consisted in care of the otitis media, a mixed diet and ferrum reductum (gr. II, t. i. d.). The patient improved and gained in weight; the spleen diminished in size so that, on discharge from the hospital October 2, 1919, the edge was only palpable. The size of the liver remained unchanged.

This case is an example of a full-term child, properly fed, without rickets but having a mild infection, who showed, with a moderate general glandular enlargement and moderate enlargement of the spleen and liver, a mild secondary anemia with leucocytosis, a high percentage of lymphocytes, and a few immature lymphoid and myeloid cells.

CASE 6.—J. S., a white infant, aged 20 months, was admitted to The Harriet Lane Home June 8, 1919, because of refusal to take food.

The patient was born prematurely at the ninth lunar month and weighed five pounds at birth. He was artificially fed from birth and difficulty had always been experienced in getting him to take food. He had not received any addition to a milk mixture diet on this account. Mental development had been retarded, he had made no attempt to sit or stand and he did not seem to notice objects put before him. When seen in the out-patient department at the age of 13 months, he showed slight enlargement of the spleen, and pallor of the skin and mucous membranes. No blood examinations were made at this time. June 7, 1919, the child had a convulsion which was followed by vomiting and fever. He was admitted to the hospital on the following day.

Examination at that time showed a fairly well-nourished but underdeveloped white infant weighing 13 pounds. There was striking pallor of the skin and mucous membranes. There were no hemorrhages into the skin or mucous membranes. The child paid no attention to

TABLE I.—BLOOD CHART OF CASE 1

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Rosophilia	Normobl.	Megakobl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myleocytes	Myleoblasts	Uncluss.	Platelets	Remarks
3-18-20	3,520,000	32	+	+	0	2	0	13,600	52.0	5	1	23.5	3.0	1.0	0.3	0	0	10.0		Normal.	
3-22-20	3,140,000	33	1	12,600													
3-26-20	3,740,000	35	+	+	+	+	5	17,000	51.5	5	0	23	2.0	2.0	0.4	0	0	13.0		Normal.	Otitis and bronchitis. Temperature 99 to 103° F.

TABLE II.—BLOOD CHART OF CASE 2

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophilia	Normobl.	Megakiohl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myelocytes	Myeloblasts	Unclass.	Platelets	Remarks
9-1-20	3,840,000	65	+++	++++		0	0	8,200	36.0	0	0	49.0	.51	0.45	2.5	5.0	0	6.0		Moderately increased.
4-22-21	5,280,000	52	+	+	0	0	0	13,200	48.5	0	.5	20.5	14.0	3.5	3.5	1.0	0	0	8.5	Increased.

TABLE III.—BLOOD CHART OF CASE 3

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophils	Normob.	Megakoth.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Traps	Medocytes	Myeloblasts	Uncl.ass.	Platelets	Remarks	
5-12-20	2,363,000	27	+	+	+	1	1	17,800	36.0	0	0	42.0	3.0	.55	.56	0	0	7.0	Normal.		Bronchopneumonia. Temperature 102° F.	
5-23-20	+++	+++	+	2	1	18,400	60.0	0	0	16.0	2.4	.46	.84	.8	0	0	9.6	High normal.		Temperature 103° F.

TABLE IV.—BLOOD CHART OF CASE 4

[illegible]

TABLE V.—BLOOD CHART OF CASE 5

[illegible]

his surroundings and did not seem to see; the eyes would not follow a bright light. He was unable to sit up or to support his head. The head was flattened posteriorly with very high forehead and prominent bosses. The fontanelles were closed. There was slight enlargement of the costochondral junctions and slight epiphyseal enlargement. There were, however, no deformities. Ophthalmological examination showed pallor of both discs, but whether this was due to primary optic atrophy or was a result of the severe anemia was not determined. The temperature on admission was 104° F. and remained elevated for 72 hours. There was no evidence of any infection to explain the fever. There was no enlargement of the lymph glands. The spleen edge was felt three fingers' breadth below the left costal margin. The liver edge extended two fingers' breadth below the right costal margin.

The spinal fluid was normal. The Pirquet and intracutaneous tuberculin (0.1 mg. O. T.) were negative. The Wassermann test was negative with blood and spinal fluid, and the mother's Wassermann test was negative. The blood showed a severe secondary anemia with leucopenia and marked relative lymphocytosis (see Table VI).

It was found extremely difficult to feed the patient. He would swallow milk when placed in his mouth, but would not swallow solid food. June 10, 1919, he was transfused with 200 c.c. of citrated whole blood from the mother. There was no marked reaction. Following transfusion the temperature fell to normal and remained so. The general condition of the patient improved and he was taught to swallow solid food and gradually made to take a mixed diet. The spleen decreased in size. The child failed entirely, however, to develop mentally. He was discharged from the hospital July 31, 1919, and reported regularly for examination. No new features developed except occasional convulsions. His spleen has remained palpable and the blindness has persisted. When last seen, July 21, 1921, the patient was well nourished and taking his diet well, but without improvement in his mental development.

The blood picture in this case is extremely interesting and for that reason will be presented in some detail. The red blood cell count, moderately reduced at first, improved under treatment and, although there were some retrogressions, finally became normal and has remained so for two years. The same may be said of the hemoglobin percentage, although the initial reduction was much more severe than that of the red blood cell count. While the anemia was most pronounced, there was a minimal grade of anisocytosis and poikilocytosis and, very rarely, slight diffuse basophilia. The variations in the white blood cell count and differential picture were extremely interesting. During the stage of most severe anemia there was a moderate leucopenia. As the anemia improved the count became normal and later, in the presence of a minor infection, there was a moderate leucocytosis. During the early stages of study at the time the anemia was severe and slight leucopenia was present, there was a most marked reduction in the percentage of myeloid cells amounting on one day to a complete absence. At this same time the lymphocytes were proportionally increased so that on the day when no myeloid cells were present 94 per cent lymphocytes were counted. Among the lymphocytes there was a good percentage of immature pathological forms presenting widely different morphological features. As the anemia improved, the percentage of lymphocytes rapidly became less, giving way to myeloid cells. When the myeloid cells began to return to the capillary circulation, they were largely immature forms so that on one day there were 19 per cent myelocytes and two per cent myeloblasts counted. These rapidly gave way to normal polymorphonuclear cells, however, and at no time again were immature myeloid cells encountered. The immature lymphoid cells were present constantly, at least in small numbers, for many months. The number of platelets present was essentially normal throughout the course of the illness.

This patient, born prematurely, and artificially fed, mentally retarded, with mild rickets and a moderate enlargement of the liver and spleen, had an anemia associated with such a

severe lymphoid reaction that it was difficult, if not impossible, to differentiate it from lymphoid leukemia when first seen. Such reactions have been reported, but they are rare; and it should be emphasized that they occur.

CASE 7.—M. van W., a white girl, aged three and one-half years, was admitted to The Harriet Lane Home August 1, 1919, for observation.

The patient was born at term and was breast fed for eight months. Following this she was given whole cow's milk and cereals and then a general mixed diet. She had never learned to walk. There was a vague history of convulsions. She had received cod-liver oil and bitter wine of iron for five months prior to admission.

Examination on admission to the hospital showed a poorly-nourished child with marked rickets. The weight was 22 pounds. The head was square, the frontal and parietal bosses prominent. The epiphyses were enlarged and there were marked deformities. There was pronounced pallor of the skin and mucous membranes. The abdomen was prominent, the muscle tone was poor, and the muscles flabby and weak. There was no enlargement of the lymph nodes. The spleen was greatly enlarged reaching the crest of the ilium. The liver was not palpable.

The urine was of low specific gravity, large in amount, with albumin but no casts. It was sterile on culture. The phenolsulphonphthalein excretion was 4 per cent for two hours. Cystoscopic examination revealed a stricture of the right ureter. The fundi oculorum were normal. The blood pressure was normal. The Pirquet and Wassermann tests were negative. X-rays of the head and long bones showed marked rickets. The blood in this case showed a moderately severe reduction in red blood cells and hemoglobin, a moderate grade of anisocytosis, poikilocytosis and basophilia, the presence of a few nucleated forms; and a white blood cell count varying from 7800 to 17,500, showing at all times a lymphoid reaction and a few immature myeloid and lymphoid cells (see Table VII).

The patient did poorly during her stay in the hospital and on August 16, 1919, was transfused without reaction with 175 c.c. of citrated whole blood. Following this the patient improved somewhat, but only temporarily. She refused food, became weaker and died October 28, 1919.

Autopsy findings.—Advanced rickets, anemia, splenomegaly; fatty liver; congenital malformation of the kidneys.

This case is that of a child three and one-half years old, born at term and properly fed who showed a marked enlargement of the spleen and an anemia with a lymphoid reaction and many immature cells of all types. A leucocytosis, although present at times, was not constant. The changes due to rickets in this child were very marked.

CASE 8.—E. D., a colored infant, aged six and one-half months, was admitted to The Harriet Lane Home March 17, 1917, because of weakness, failure to develop and jaundice.

The patient was born at term, weighed seven pounds at birth, and had been entirely breast fed. The mother noted on March 3, 1917, that the skin was becoming yellow and that the napkins were stained yellow.

Examination on admission showed a well-nourished, fairly active infant weighing 13 pounds. There was marked jaundice of the skin, sclerae and mucous membranes. The head was symmetrical and had prominent frontal bosses. The anterior fontanelle was widely open. The mucous membranes were pale. A blowing systolic murmur was heard over the heart. There was no enlargement of the epiphyses nor deformities of the thorax, spine or extremities. There was no general enlargement of the lymph glands. The spleen extended two fingers' breadth below the right costal margin.

The Pirquet and Wassermann tests were negative. The blood picture showed a very severe reduction in red blood cells and hemoglobin, pronounced anisocytosis, poikilocytosis and basophilia,

TABLE VI.—BLOOD CHART OF CASE 6

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophilia	Normobl.	Megalobl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myelocytes	Myeloblasts	Unclass.	Platelets	Remarks
6-8-19	2,400,000	20	+	0	0	0	0	7,600	5.0	0	0	60.0	0	17.0	17.0	...	0	0	1.0	Normal.	Fever. (Temp. 104° F.)
6-9-19	0	0	0	0	0	0	6,800	0	0	0	67.0	0	27.0	4.0	...	0	0	2.0	Normal.
6-10-19	+	+	0	0	0	0	5,400	7.0	0	0	50.0	0	22.0	13.0	...	4.0	0	4.0	Normal.	Transfusion with 200 c. c. citrated blood.
6-11-19	2,900,000	38	+	+	0	0	0	6,400	8.0	0	0	53.0	0	12.0	4.0	...	19.02	2.0	2.0	Slightly diminished.
6-12-19	3,700,000	45	+	+	0	0	0	8,800	30.0	0	0	35.0	0	8.0	15.0	...	1.0	0	11.0	Diminished.	Temperature normal.
6-13-19	3,500,000	50	+	+	0	0	0	7,000	40.0	0	1.0	37.0	0	3.0	13.0	...	0	0	6.0	Increased.
6-14-19	4,600,000	55	+	+	0	0	0	12,500	38.0	0	0	46.0	0	11.0	3.0	...	0	0	2.0	Diminished.
6-17-19	3,800,000	50	+	+	0	0	0	14,500	41.0	0	0	54.0	0	2.0	3.0	...	0	0	0	Normal.
6-20-19	4,496,000	12,700
6-25-19	4,976,000	66	+	+	+	0	0	9,400	59.0	1.0	0	31.0	0	6.0	1	2	0	0	0	Increased.
7-6-19	2,944,000	52	+	+	0	0	0	7,240	47.0	2.0	0	36.0	0	7.0	1	6.0	0	0	1	Moderately increased.
7-13-19	4,680,000	75	+	+	0	0	0	6,480	43.0	2.0	0	47.0	0	2.0	0	5.0	0	0	2	Diminished.	Reduced iron begun.
7-22-19	+	+	0	0	0	51.0	0	0	33.0	0	2.0	1	7.0	0	0	6	Normal.	
7-31-19	5,240,000	76	+	+	0	0	0	13,200	55.0	3.0	0	29.0	0	1.0	2	2.0	0	0	8	Diminished.
8-15-19	5,212,000	74	+	+	0	0	0	12,000	46.0	1.0	0	35.0	0	11.0	2.02	2.0	0	0	3.0	Normal.
9-2-19	5,784,000	76	0	0	0	0	0	14,400	50.0	2.0	0	23.0	0	12.0	2.03	3.0	0	0	8.0	Diminished.
10-6-19	0	0	0	0	0	0	13,400	45.0	.5	0	27.5	14.0	1.5	4.52	5.0	0	0	4.5	Diminished.
11-25-19	5,920,000	84	+	+	0	0	0	11,200	46.0	.5	0	26.5	12.0	3.5	1.55	5.0	0	0	4.5	Normal.
12-18-19	4,850,000	82	17,500
2-12-20	85	0	0	0	0	0	18,000	42.0	1.5	0	34.0	8.5	3.5	3.06	6.0	0	0	1.5	Increased.
3-29-20	72	0	0	+	0	0	9,500	38.5	.5	0	47.5	3.0	2.5	3.02	5.0	0	0	2.5	Normal.
4-19-20	17,600
4-20-20	4,352,000	76	+	+	0	0	0	12,600	Normal.
5-5-20	0	0	0	0	0	0	High normal.
7-21-20	70	+	+	0	0	0	15,000	40.0	1.5	0	39.5	3.0	0.5	.52	5.0	0	0	7.5	Normal.

TABLE VII.—BLOOD CHART OF CASE 7

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophilia	Normobl.	Megalobl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myelocytes	Myeloblasts	Unclss.	Platelets	Remarks
8-1-9	2,460,000	43	+	+++	+++	2	1	13,350	35.06	0	33.0	9	9.03	0.6	0.1	0.2	0	5.0	Normal.		
8-13-19	2,592,000	41	++	++	0	3	0	17,500	70.02	0	18.0	0	4.0	0.2	0.1	0	0	3.0	Slightly diminished.		8-16-19. Transfusion 175 c. c. citrated whole blood.
8-20-19	3,560,000	60	0	0	0	0	0	9,000	26.04	0.5	37.0	0	8.0	1.05	0	0	1.0	13.0	Diminished		
9-1-19	2,960,000	70	+++	+	+	0	1	14,000	31.01	0	36.0	0	5.05	0.6	0	0	0	16.0	Diminished.		
9-26-19	2,496,000	28						13,900													
10-10-19	2,336,000	36						7,800													
10-17-19		38	0	+	+	0	2	9,300	58.82	.4	0	17.6	.4	.86	.44	.8	1.6	0	7.2	Normal.	

and very many nucleated forms; and a normal white cell count showing the presence of immature myeloid and lymphoid cells. The fragility of the patient's red blood corpuscles to hypotonic salt solutions was within the normal limits and identical with those of a normal individual.

The patient was placed on a mixture of cow's milk, barley water and sugar, with the substitution of breast milk when available, and cereals. He was transfused nine times with citrated whole blood in amounts varying from 50 to 115 c.c., a total of 810 c.c., between March 27, and July 16, 1917. Improvement was rapid; the jaundice disappeared and the spleen diminished in size. By August 23, 1917, the patient was taking a mixed diet and had gained a pound and a half in weight. He has remained well since that time.

This case is that of an infant born at term and breast fed who had very mild rickets and some jaundice, and showed, with a moderate enlargement of the spleen and liver, an anemia with many immature cells of all types. There was no leucocytosis.

CASE 9.—C. S., a colored infant, aged 14 months, was admitted to The Harriet Lane Home February 4, 1921, because of anemia.

Birth was at term. The patient was breast fed for one month and then given condensed milk, malted milk and whole milk formulae. At the time of admission he was taking a mixture consisting of 3 parts milk and 1 part water, with cereal twice a day. The patient had begun to stand with support but could not walk.

Examination on admission showed a fairly well-nourished colored infant weighing 15 pounds. The head was asymmetrical with a projection of the left frontal region and a corresponding depression on the right side. There was slight enlargement of the epiphyses but no deformities. The mucous membranes were pale. The abdomen was distended. There was slight but definite general enlargement of the lymph nodes. The spleen was felt three fingers' breadth below the left costal margin and the liver extended three fingers' breadth below the right costal margin.

X-rays of the long bones showed rickets. The Pirquet and Wassermann tests were negative. The temperature was normal. The blood in this case showed a moderately severe reduction in the red blood cells, a very pronounced diminution in hemoglobin, marked anisocytosis, poikilocytosis, and basophilia, and very many nucleated forms; a leucocytosis, sometimes very high, a severe lymphoid reaction, and the presence at most times of immature lymphoid and myeloid elements (see Table IX).

The patient was given a diet of whole milk, cereals, and meat, with the addition of broth, green vegetables, etc., cod-liver oil, and reduced iron (gr. II, t.i.d.). Progress was slow; the patient developed numerous infections (otitis media, rhinitis, varicella, nasal diphtheria, impetigo). On April 13 he was transfused, without reaction, with 100 c.c. citrated whole blood, and on April 29, with 100 c.c. citrated whole blood. The patient improved somewhat and was discharged May 20, 1921.

This case is an example of an infant born at term, properly fed, and having rickets, who showed, with slight general enlargement of the lymph nodes and a moderate enlargement of the spleen and liver, an anemia with leucocytosis, lymphoid reaction and immature cells. It may be compared with Case 8 and Case 10, in which no increase in the total white blood cell count was observed; and with Case 7 in which at different times a leucopenia and a leucocytosis, respectively, were present.

CASE 10.—E. B., a colored infant, aged 20 months, was brought to The Harriet Lane Home May 3, 1921, because of a "lump in the side."

The patient was born at the 8th month and was one of twins. Breast feeding was employed until the 8th month, since which time the

patient has received a mixed diet of milk, vegetables, cereal, eggs and orange juice. The mother had noted the lump in the left side for three weeks, and she said the child had seemed weak.

Examination on admission showed a small, under-nourished infant weighing 12½ pounds. The head was of normal shape and size. The conjunctivae and mucous membranes were pale and on the surface of the hard palate there were a number of hemorrhages. The thorax showed rachitic deformity. The epiphyses of the long bones were somewhat enlarged. The cervical, axillary, inguinal and epitrochlear lymph nodes were enlarged. The abdomen was distended, due chiefly to the large spleen which filled the entire left flank. The liver was slightly larger than normal.

The Pirquet and Wassermann tests were negative. The blood showed a moderate reduction in the number of red blood cells, a more pronounced diminution in hemoglobin, some poikilocytosis and anisocytosis, a most marked degree of diffuse basophilia, and relatively many nucleated red blood cells; a mild lymphoid reaction and many immature myeloid and lymphoid cells with a normal total white blood cell count (see Table X).

The patient was given a mixed diet, cod-liver oil and reduced iron. On May 14, 125 c.c. of citrated whole blood was given with a moderately severe reaction. The patient up to the present time has shown but slight improvement.

This patient, a premature infant with rickets, showed in the presence of slight enlargement of the lymph glands, marked enlargement of the spleen, and slight enlargement of the liver, an anemia presenting a mild lymphoid reaction, many immature cells and no leucocytosis. A similar picture was seen in Case 8, an infant born at term.

When these cases of anemia in infants were examined in detail, it was seen that, although some of the features presented were common to all the patients, others varied within wide limits. Diminution in the number of red blood cells and in the hemoglobin percentage, anisocytosis, poikilocytosis and basophilia were present in all, and in general the severity of these changes varied with the severity of the anemia. All of the patients also showed at least a slight relative increase in cells of the large mononuclear-transitional cell group. Variations in the number and character of the platelets present from time to time were essentially the same in all cases. However, the degree of anemia, the total white blood cell count, the differential white blood cell formula, the presence of immature cells, enlargement of the spleen, liver and lymph nodes, the presence of rickets, the signs of prematurity, and the kind of feeding employed varied within wide limits. An analysis of these findings and their relation to each other permits definite conclusions.

The blood pictures in these cases showed three major types of reaction, or various combinations of them. Pure examples of them were not commonly encountered, and the cases presented here may not be classified according to this grouping. One typical example of each reaction has been presented, however, and the other cases have been chosen to illustrate different degrees and various combinations of them. The three main types of reaction, which were added to those features already presented as more or less common to all cases, were:

Type I.—The blood changes resembled somewhat those seen in simple secondary anemia of adults; that is, there were no

TABLE VIII.—BLOOD CHART OF CASE 8

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophilia	Normobl.	Megalobl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myelocytes	Myeloblasts	Unclass.	Platelets	Remarks
3-23-17	1,000,000	20	++++	++++	+++	Numerous.	Numerous.	8,160	48.0	0	0.5	37.5	0	2.0	8.0	0	4.0	0	0	Normal.
4-6-17	1,800,000	31	6,650	50	0	0	37.5	3.53	3	2.5	0
7-18-17	3,000,000	50	7,000	After nine transfusions.

TABLE IX.—BLOOD CHART OF CASE 9

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophilia	Normobl.	Megalobl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myelocytes	Myeloblasts	Unclass.	Platelets	Remarks	
2-4-21	2,992,000	20	76,000	
2-6-21	++++	++	++	37	8	54,000	31.6	.4	0	23.2	0	36.0	6.4	0	0	2.4	Normal.	
2-8-21	2,560,000	20	+++	+++	++++	Very numerous.	...	32,000	25.0	1.0	1.0	26.0	0	33.0	8.0	0	0	3.0	Mod. diminished.	
2-12-21	2,168,000	21	++++	+++	0	0	7	16,400	10.8	.4	0	4.4	0	8.8	3.64	0	71.6	Normal.	
2-18-21	2,552,000	35	+++	+++	++++	0	0	12,000	65.5	2.5	0	10.0	4.0	6.5	6.0	1.0	0	4.5	High normal.	
2-24-21	2,424,000	36	+++	++++	0	Few.	0	12,800	17.0	5.0	1.0	33.0	5.0	6.0	5.0	3.0	0	25.0	High normal.	
3-16-21	+++	+++	++++	0	1	25,920	50.4	1.6	.4	15.6	3.6	13.6	4.8	0	0	10.0	Normal.	
3-31-21	2,784,000	46	+++	++	++++	20	0	22,400	20.4	.8	0	36.4	12.4	2.8	20.0	1.2	0	6.0	High normal.	
4-13-21	2,648,000	48	++++	Sickle cells.	++++	7	1	25,400	50.0	.8	.4	8.4	19.2	2.8	2.4	2.0	0	0	14.0	Normal.
4-14-21	2,048,000	60	++++	++++	+++	11	0	24,200	48.0	.4	.4	27.6	10.0	1.6	5.6	1.6	0	4.8	Low normal.	Transfusion 100 c. c. citrated blood 4-3-19.	
4-21-21	2,448,000	46	+++	+++	+++	Very rare.	0	19,480	47.5	1.0	0	26.0	6.0	0	6.0	5.5	0	0	8.0	High normal.
4-30-21	2,624,000	52	+++	+++	+++	3	0	28,240	40.0	2.0	.5	42.0	2.0	1.5	4.5	4.5	0	0	3.0	Normal.
5-11-21	2,248,000	50	+++	+++	++	0	0	21,000	55.0	1.0	.5	33.5	2.5	2.0	2.5	2.5	0	0	.5	High normal.	Blood taken just before transfusion of 100 c. c. citrated blood.
5-14-21	2,536,000	50	++	++	+	0	0	15,720	39.0	.5	0	44.5	.5	0	9.5	3.5	0	0	2.5	High normal.	Blood taken just before transfusion of 100 c. c. citrated blood.
5-17-21	2,968,000	60	++	++	++	0	0	15,920	45.0	2.5	0	36.5	2.5	0.5	6.0	3.0	0	0	4.0	High normal.

TABLE X.—BLOOD CHART OF CASE 10

Date	R. B. C.	Hb. %	Aniso.	Poikilo.	Basophilia	Normobl.	Megalobl.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	P. L.	L. M.	Trans.	Myelocytes	Myeloblasts	Unclass.	Platelets	Remarks
5-6-21	4,064,000	50	++	+	++++	9	10,860	44.0	4.5	.5	.5	23.0	1.5	2.5	11.0	8.0	0.3	0	2.0	Low normal
5-14-21	3,904,000	40	++	++	++++	11	4	10,240	35.6	.0	.8	28.4	6.0	4.4	9.2	6.8	6.0	0	2.8	Low normal
5-18-21	4,072,000	50	+	+	+++	1	0	10,200	50.5	1.5	.0	13.5	2.0	2.5	9.5	8.5	7.5	0	4.5	Low normal
6-9-19	3,408,000	40	+	+	+++	14	3	15,200	39.0	1.5	.0	32.5	5.5	4.5	3.0	6.5	4.5	0	3.0	Low normal

immature forms and no increase of lymphocytes. Case 1 was a typical example of this reaction. Cases 2 and 3 showed essentially the same blood pictures, but had some increase in lymphocytes and therefore stood midway between Case 1 and the second type of reaction.

Type II.—The blood showed, in addition to the changes of Type I, an increase in the relative proportion of lymphocytes. Case 6 was an example of this type of reaction. Cases 4 and 5 also showed an increase in lymphocytes as the outstanding feature, but had in addition a few immature cells. They, therefore, stood midway between Case 6 and the third type of reaction.

Type III.—The blood showed, as the most pronounced change, immature cells of all types. There was also in these

The age of the patient apparently bore no relationship to the blood picture encountered, or to any of the chief clinical features of the case.

Prematurity was present in six of the cases studied. In these cases no constant quantitative or qualitative blood changes were found. Also different degrees of splenic enlargement were present. It may be said, therefore, even from this small series of cases, that although secondary anemia is frequently observed in infants born prematurely, the changes in the blood are not characteristic, and the degree of splenic enlargement is not constant.

The feeding of these infants was entirely from the breast in one case and either by combination of breast and artificial feeding or by artificial feeding alone in the others. There

TABLE XI

Case	Age	Prematurity	Feeding	Rickets	Enlargement of lymph glands	Enlargement of spleen	Enlargement of liver	Infection	Other conditions	Blood
1	9 mos. 7th mo.	mo.	Art.	Mild	0	Marked	Slight	Otitis media bronchitis	Moderately severe, simple secondary anemia and leucocytosis.
2	11 mos. 7th mo.	Br. and art.		Mild	0	Slight	0	0	Mild, simple secondary anemia, slight leucocytosis, and moderate lymphoid reaction.
3	8 mos. 7th mo.	Br. and art.		Moderate	Slight	0	0	Broncho-pneumonia	Severe secondary anemia with leucocytosis and moderate lymphoid reaction.
4	9 mos. 6th mo.	Art.		Mild	0	Slight	0	0	Moderately severe, secondary anemia with leucocytosis, a moderate lymphoid reaction and an occasional immature myeloid cell.
5	34 mos.	0	Br. and art.	0	Moderate	Moderate	Moderate	Otitis media	Mild secondary anemia and leucocytosis, a moderate lymphoid reaction many immature lymphoid and a few immature myeloid cells.
6	20 mos. 9th mo.	Art.		Mild	0	Moderate	Moderate	Temp. 104°F. Unexplained	Blindness and idiocy	Severe anemia and marked lymphoid reaction with many immature cells of all types.
7	42 mos.	0	Br. and art. and mix.	Severe	0	Very Marked	0	0	Cystic kidneys	Moderately severe anemia, both leucopenia and leucocytosis, lymphoid reaction, and immature cells of all types.
8	6½ mos.	0	Br.	Very mild	0	Moderate	Moderate	0	Very severe anemia, no leucocytosis, and immature cells of all types.
9	14 mos.	0	Br. and art.	Mild	Slight	Moderate	Moderate	Otitis media Varicella Diphtheria Impetigo	Moderately severe anemia with leucocytosis, often very high, lymphoid reaction and immature cells of all types.
10	20 mos. 8th mo.	Br. and art.		Moderately severe	Slight	Marked	Slight	0	Moderately severe anemia, no leucocytosis, slight lymphoid reaction, and immature cells of all types.

cases some increase in the percentage of lymphocytes. Cases 7, 8, 9 and 10 might be assigned to this group. When there was a leucocytosis, as in Cases 7 and 9, the picture presented was that commonly spoken of as von Jaksch's anemia.

It would seem, therefore, that the simple anemias of infants differed in blood picture from those of adults chiefly by reason of two special reactions, lymphocytic increase and the presence of immature cells. Either one of these might be present alone and in different degrees, or both be found, the relative importance of one toward the other also the subject of wide variation.

In Table XI the salient clinical features and a summary of the blood picture in each case are presented. Study of this chart reveals several points worthy of note.

was no relationship between any particular type of feeding and the character of the anemia or other clinical features present, although the data on this point are perhaps too meagre to permit definite conclusions. Rickets was present in all but one patient in this series, and this child was nearly three years of age. The striking feature of the rickets found in these patients was that, although slight enlargement of the epiphyses and costochondral junctions was present in the majority, pronounced rachitic changes in the cranial bones were evident. No relationship between the degree of rickets and the severity of the anemia or the type of blood reaction could be demonstrated. Similarly, the severity of the rickets did not parallel the degree of enlargement of the spleen, liver, or lymph nodes.

Enlargement of the lymph nodes was found in four cases, but was independent of the blood reaction, and the presence, absence, or amount of enlargement of the spleen and liver.

Enlargement of the spleen was a factor of greatest variation, varying from a spleen of normal size to those showing great enlargement. One could not predict from the size of the spleen what the blood picture would be, for severe anemia with immature cells was encountered with slight splenic enlargement, and simple anemia without abnormal cells occurred with a huge spleen. The fact that the degree of splenic enlargement could not be correlated with the severity of the rickets present may again be emphasized.

Enlargement of the liver was not marked in any case. When present, it was found with enlargement of the spleen; splenomegaly, however, occurred independently of hepatomegaly.

Various infections, so common in this class of patients, may have temporarily influenced the blood reaction, but in none of these patients could the anemia be attributed solely to infection. There was no peculiar type of blood reaction in them, nor any constancy in the degree of the enlargement of the spleen or lymph nodes.

Leucocytosis was a very variable feature and was in some cases apparently associated with an acute infection. The total number of leucocytes, however, bore no constant relationship to the other features of the case.

From the foregoing analysis of these cases it is seen that in infants with anemias, enlargement of the spleen is very common and is often very marked; and enlargement of the liver and lymph nodes is frequently encountered. The importance of this enlargement in diagnosis and prognosis is very much less than in the adult. In these cases great variation in the blood picture may occur. Although various grades of anisocytosis, poikilocytosis, basophilia and a relative increase in cells of the large mononuclear-transitional cell group are found in all, other qualitative changes vary markedly both in regularity of occurrence and degree of severity. The blood may show only the changes of a simple secondary anemia as seen in adults; it may show, in addition to this, different degrees of lymphocytosis sometimes so marked that the differentiation from lymphoid leukemia may be difficult at first; it may show the presence of immature blood cells of all types, the so-called von Jaksch's anemia; or it may present any gradation between these three major types. In the presence of any of these qualitative changes there may or may not be a leucocytosis. The occurrence of the different blood pictures appears to be wholly unrelated to the other findings. It is not possible to predict from the physical examination of the child what the character of the blood reaction will be. The type of reaction shown by the blood does not seem to be dependent upon the degree of anemia, the presence, absence, or severity of rickets, or to be influenced by prematurity or birth at term.

The reaction on the part of the infant with enlargement of the spleen, liver and lymph nodes, and with changes in the blood picture as described above constitutes a fairly definite

symptom complex, peculiar to the first few years of life. This symptom complex has been given special names such as "infantile splenic anemia," anemia infantum pseudoleukemica," and "von Jaksch's anemia" and considered as a special disease or some atypical kind of leukemia. From examination of the case histories given above it would seem that this is not justified. This symptom complex has not been shown to be a disease *sui generis* and is not as yet entitled to a special name. Any name which suggests leukemia is especially unfortunate, for cases such as are presented above are not leukemic in any stage and bear no relationship to leukemia. Hence, the use of such names as "anemia infantum pseudoleukemica," and "von Jaksch's anemia" which by authority of his original publication implies a relationship to leukemia, should be discontinued. Whatever combination of anemia, total white blood cell count, differential formula, enlargement of the spleen, liver or lymph nodes is encountered in these cases, or whatever may be the relative severity of any one or more of these findings, the significance is the same and permits only the same interpretation. This symptom complex can best be interpreted as the peculiar type of reaction on the part of the infant to the presence of a factor producing secondary anemia.

The reason for the enlargement of the spleen, liver, and lymph nodes, a lymphocytosis, and the appearance of immature cells, both of the red and white blood cell series in the peripheral circulation, and the marked variation in the presence and degree of these signs so frequently encountered in infants with anemia is not clear. The fact that this symptom complex is found in association with so many different conditions (prematurity, severe nutritional disorders, rickets, tuberculosis, syphilis, malaria, leishmaniasis and other acute and chronic infections) would make it appear probable that these various manifestations are brought about by no special etiologic factor. It seems quite probable that the infantile hematopoietic system is more labile than that of adults, and that in the presence of an anemia, immature cells of all types enter the peripheral circulation more readily and the other changes mentioned are more easily brought about. If this be true, any etiologic agent which would produce in the adult merely a simple secondary anemia (reduction in the number of red blood cells and hemoglobin alone) may, in the infant, result in more severe and striking changes. The possibility must be also mentioned that these changes are brought about by the presence of some special factor or influence that is operative in infants but not in adults. Among the conditions peculiar to the period of life in which this symptom complex is observed, may be mentioned the immature state of the hematopoietic system accompanying premature birth, the peculiar dietetic influences present in infants, and rickets. Although we have shown that the symptom complex described is frequently seen in association with these conditions, we have no evidence that these peculiar conditions are entirely responsible for the reaction observed. Rickets, especially, or its underlying cause has been suggested by many as the etiologic agent in question. The evidence at hand, however, per-

mits us to state only that the two conditions (rickets and anemia) are frequently seen in association with each other. Whatever the underlying cause may be, the symptom complex which we have described remains a secondary anemia, peculiar only in the variability of the infantile response.

CONCLUSIONS

1. In infants with anemia, enlargement of the spleen is frequent, and enlargement of the liver and lymph nodes is fairly common. These findings alone are of no specific diagnostic or prognostic importance.

2. The infantile hematopoietic system frequently reacts to anemia with a relative lymphocytosis, by throwing out immature blood cells, or with both of these qualitative changes in varying grades of severity. Any of these reactions may be present with or without a leucocytosis, and may have no serious significance.

3. The presence, absence, or degree of splenomegaly, hepatomegaly, or general enlargement of the lymph nodes, the severity of the anemia, the total white blood cell count, or the type of qualitative changes in the blood, bear no constant relation to each other.

4. This symptom complex has not been shown to be a disease *sui generis* and all variations of it are probably merely an infantile response to some agent producing secondary anemia. It is not yet entitled to any special name, especially one that suggests a relationship to leukemia.

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PREGNANCY AND LABOR IN YOUNG PRIMIPARÆ

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In spite of the fact that most text-books of obstetrics contain little information upon the subject, there is a prevalent opinion that pregnancy and labor are attended by greater danger in young girls than in older women. Williams¹ differs from this view and states that labor in the girl of 16 or less is no more serious than in the women of more mature years. In order to test the correctness of this view I have collected and analyzed all labors in girls of 16 years of age or less which have occurred in the obstetrical service of The Johns Hopkins Hospital.

Varnier² has compared the average duration of labor in 100 primiparæ of less than 20 years of age with that in a similar number of patients between the ages of 20 and 30.

He found the average duration was 13 hours, 5 minutes in the former as compared with 13 hours, 28 minutes in the latter. In all of the patients in both groups the pelvis was normal and the child presented by the vertex.

Gache,² of Buenos Aires, analyzed 91 cases of labor occurring in girls between the ages of 13 and 16. In 84 patients the pelvis was normal. Of the 91 patients, 78 were delivered spontaneously and 13 by operative measures. He estimated that the average duration of labor was 24 hours and that the children averaged 3039 grams in weight, and concluded that, so far as he could ascertain, the age of the patient is practically a matter of indifference.

Bondy⁴ has reported 69 labors between the ages of 14 and 16, of which 12 resulted in the birth of premature children. In the 57 patients delivered at term he believed that labor was prolonged in 19 instances.

From the first 10,000 admissions to the obstetrical service of The Johns Hopkins Hospital I have collected the details of 500 labors occurring in girls between the ages of 12 and 16 inclusive. Since most of the abortions are admitted to the gynecological service, the few that appear in our records have not been considered, as they would give no accurate picture of the frequency of this complication.

Table 1 presents an analysis of the age at which delivery took place in each of the two races, and shows that there are more than twice as many blacks as whites in the series. As less than 45 per cent of all patients admitted to the service are blacks, this unusual predominance of colored young primiparæ may be regarded as an index of the incidence of precocious sexual relations in that race.

TABLE 1

Age	White	Black	Total
12	0	2	2
13	1	6	7
14	18	28	46
15	45	95	140
16	96	209	305
Total	160	340	500

Pelvis.—In every patient in the series the pelvis was measured both externally and internally and the results are shown in Tables 2 and 3.

In the 160 white patients contracted pelvis were noted in 21, a percentage of 13.12. The generally contracted and the funnel were the most frequent types noted, making up 16 of the 21 cases.

Williams,⁵ in a series of 1313 white women, irrespective of age, found that the incidence of contracted pelvis was 13.33 per cent, and stated that the funnel and the generally contracted were the two types most frequently encountered, the two making up 125 of his 175 cases. On comparing our figures with his, it is seen that the incidence of contracted pelvis is practically the same in both series, and that the types of pelvic contraction most frequently noted were identical. In other words, the white girl of 13 to 16 years of age has as large a pelvis as her older sister.

In the 340 blacks in our series the incidence of pelvic contraction was 60.58 per cent. The two most frequent types encountered were the generally contracted and the generally contracted rhachitic, the two making up 140 of our 206 cases. Williams⁶ found 312 contracted pelvis in 902 colored women of all ages, a percentage of 40.93. Of these the generally contracted and the generally contracted rhachitic made up 258 of his 312 cases. Thus it is seen that in colored young

primiparæ contractions of the pelvis occur 50 per cent times more frequently than in colored women of more mature years. This is to be expected when it is remembered that casual observation seems to indicate that the black woman in Baltimore reaches physical maturity later than does the white. Our figures are in accord with those of Williams that the two types of contracted pelvis most frequently noted in the black are the generally contracted and the generally contracted rhachitic.

Abnormal Pregnancy.—Table 4 shows the complications of pregnancy met with in the series of 500 cases.

TABLE 2

CLASSIFICATION OF Pelves IN 160 WHITE YOUNG PRIMIPARÆ

Age	Normal pelvis	Contracted pelvis					Total
		Generally contracted	Simple flat	Funnel	Gen. cont. funnel	Total cont. pelvis	
13	1	0	0	0	0	0	1
14	14	1	0	2	1	4	18
15	37	5	0	2	1	8	45
16	57	3	2	3	1	9	96
Total	139	9	2	7	3	21	160

TABLE 3

CLASSIFICATION OF Pelves IN 340 BLACK YOUNG PRIMIPARÆ

Age	Normal pelvis	Contracted pelvis							Total
		Generally contracted	Simple flat	Gen. cont. rhachitic	Flat rhachitic	Funnel	Gen. cont. funnel	Coxalge	
12	0	2	0	0	0	0	0	0	2
13	0	3	0	3	0	0	0	0	6
14	8	11	0	4	0	2	3	0	28
15	41	16	0	16	1	9	12	0	95
16	85	51	1	34	1	12	24	1	209
Total	134	83	1	57	2	23	39	1	340

TABLE 4

ABNORMAL PREGNANCIES

	Preeclamptic toxæmia	Eclampsia	Nephritic toxæmia	Syphilis	Pyelo-nephritis	Typhoid	Menses	Total
White	2	9	1	1	0	0	0	13
Black	8	7	0	25	1	1	1	43
Total	10	16	1	26	1	1	1	56

At first glance it appears that there was an abnormally high incidence of preeclamptic toxæmia and eclampsia, 10

of the former and 16 of the latter. However, five of the cases of the former and 14 of the latter were not treated in the prenatal clinic but were referred to the service because of the existence of the disease. Consequently, no such incidence of these complications should be expected to obtain in the average run of young primiparae.

It is noted that there were 26 cases of syphilis in the series, 25 being in black women and 1 in white. This is a much lower incidence of the disease than that reported from this clinic by Williams.* The discrepancy can be explained not only on the grounds that in the young girl syphilis would not be expected to be present so frequently, but also because of the fact that more than half of the patients in the series passed through our hands before the introduction of the routine Wassermann determination and, therefore, it may be assumed that many cases of infection escaped detection.

Premature Labor.—Of the entire series of 500 cases 58, or 11.6 per cent, terminated in premature labor. When divided according to race, it is seen that this termination occurred in 17 of the 160 whites and in 41 of the 340 blacks, an incidence of 10.52 and 12.05 per cent, respectively.

In the majority of cases in both races the cause of premature termination could not be determined. In the blacks syphilis was the most important ascertainable etiological factor, and it seems safe to surmise that, had the more recent methods of diagnosis been applied throughout the series, many of the cases classified as undetermined would have shown that syphilis was the causative factor.

TABLE 5
PREMATURE LABORS

	Undetermined	Syphilis	Eclampsia	Prem. sep. placenta	Twins	Monsters	Measles	Typhoid	Total
White .	11	1	2	0	2	1	0	0	17
Black..	24	11	1	2	1	0	1	1	41
Total..	35	12	3	2	3	1	1	1	58

Duration of Labor.—Of the 442 patients delivered at term the duration of labor was accurately recorded in 430—138 whites and 292 blacks. The 12 remaining patients were delivered by Cæsarean section or *accouchement forcé* before the cervix had become fully dilated.

Tables 6 and 7 show the average duration of term labor in the two races. From Table 6 it is seen that the average duration of labor for the entire series of 138 white girls is 15 hours, 44 minutes. However, when the pelvis is normal the average falls to 15 hours, 10 minutes; on the other hand, when the pelvis is contracted, labor is 4 hours, 42 minutes, longer than when it is normal.

The average duration of labor in the 292 blacks is 16 hours, 40 minutes. This increase may be assumed to be due to the greater frequency of contracted pelvis in that race; as, when the pelvis is normal, the average duration is 14 hours, 40

minutes, as compared with an average of 18 hours, 7 minutes, when the pelvis is contracted.

It is usually stated that the average duration of labor in primiparae, irrespective of age, is 18 hours. G. Veit⁷ places it at 20 hours. Accepting the lower figure as correct, it is seen that the young primipara has a labor shorter by 2 hours, 16 minutes in the white and 1 hour, 20 minutes in the black race.

TABLE 6
AVERAGE DURATION OF LABOR—WHITE

Age	Total		Normal pelvis		Contracted pelvis	
	No. cases	Average duration	No. cases	Average duration	No. cases	Average duration
13	1	10h. 12m.	1	10h. 12m.	0	0
14	15	20h. 02m.	11	18h. 48m.	4	23h. 24m.
15	40	15h. 15m.	33	15h. 27m.	7	14h. 15m.
16	82	15h. 16m.	76	14h. 35m.	6	24h. 06m.
Total....	138	15h. 44m.	121	15h. 10m.	17	19h. 52m.

TABLE 7
AVERAGE DURATION OF LABOR—BLACK

Age	Total		Normal pelvis		Contracted pelvis	
	No. cases	Average duration	No. cases	Average duration	No. cases	Average duration
12	2	18h. 03m.	0	0	2	18h. 03m.
13	5	17h. 20m.	0	0	5	17h. 20m.
14	25	19h. 44m.	8	18h. 50m.	17	20h. 10m.
15	82	16h. 48m.	39	13h. 53m.	43	19h. 27m.
16	178	16h. 09m.	75	14h. 43m.	103	17h. 15m.
Total....	292	16h. 40m.	122	14h. 40m.	170	18h. 07m.

More precise information as to the duration of labor may be gained by the "modal" method. This is shown in Tables 8 and 9. The shaded blocks represent the total number of term labors divided into groups of two hours each, while the lines represent the labors occurring in patients with normal pelvises. Inspection of these tables shows that in both races the most frequent duration of labor in the entire series, as well as in those with normal pelvises, is between 10 and 12 hours. Unfortunately, no comparable statistics concerning the duration of labor in older women are available, but it is not believed that they would show a shorter duration than that observed in young primiparae.

It is interesting to note that, in spite of the much higher incidence of contracted pelvis in the blacks, the most frequent duration of labor is the same as for the whites. The explanation is, as will be shown later, that the babies of the former are smaller, and, therefore, are less likely to be disproportionate to the size of the pelvis.

Delivery.—Of the 442 deliveries at term, 379 were spontaneous and 63 were operative, an incidence of 85.74 and 14.26 per cent respectively. Of the latter 18 were in whites and 45 in blacks. Notwithstanding the apparently greater frequency of operative interference in the blacks, the determination of the percentage frequency shows that the incidence is approximately the same in both races, being 12.95 per cent for the whites and 14.85 per cent for the blacks.

TABLE 8

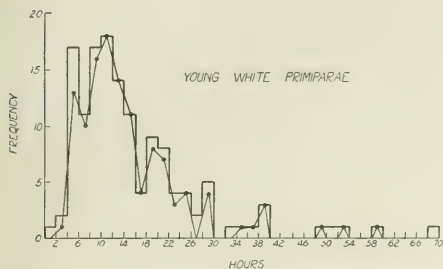
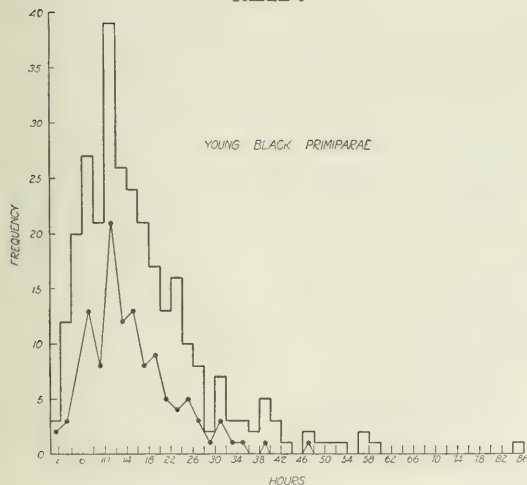


TABLE 9



Operations.—The types of operations are shown in Table 10. While the incidence of pelvic contraction was 60.58 per cent in the blacks, only 17 of the 45 operations were done because of pelvic dystocia. Of these, 10 were Cæsarean sections, 4 pubiotomies and 3 craniotomies. In the whites only two operations were necessitated by the pelvis, one Cæsarean section and one high forceps.

Weight of the Child.—In casting about for an explanation for the relatively short labor in very young primiparae it seemed possible that it might be afforded by the smaller size of the children. Accordingly the material was studied from

that point of view. Tables 11 and 12 show the average weight of the children in both races. In the series of 143 whites delivered at term the average weight of the children was 3181 grams. When classified according to the pelvis of the mother, the average in the 126 patients with normal pelvis was 3200 grams, while in the 17 with contracted pelvis it was 3045 grams, a difference of 155 grams in favor of the child born of a mother with a normal pelvis. The average weight of

TABLE 10

OPERATIONS

	Low forceps	Mid forceps	High forceps	Breach extraction	Version and extraction	Cæsarean section	Pubiotomy	Craniotomy	Accouch. forc.	Induction of labor	Total
White	7	3	2	3	0	1	0	0	0	2	18
Black	18	2	2	2	1	10	4	3	1	2	45
Total ...	25	5	4	5	1	11	4	3	1	4	63

TABLE 11

AVERAGE WEIGHT OF THE CHILDREN—WHITE

Age	Total		Normal pelvis		Contracted pelvis	
	No. cases	Av. weight	No. cases	Av. weight	No. cases	Av. weight
13	1	2700	1	2700	0	0
14	15	3063	12	3030	3	3199
15	42	3171	34	3213	8	2994
16	85	3106	79	3239	6	3037
Total....	143	3181	126	3200	17	3045

TABLE 12

AVERAGE WEIGHT OF THE CHILDREN—BLACK

Age	Total		Normal pelvis		Contracted pelvis	
	No. cases	Av. weight	No. cases	Av. weight	No. cases	Av. weight
12	2	2830	0	0	2	2830
13	5	3030	0	0	5	3030
14	24	3034	8	3211	16	2943
15	82	3043	39	3062	43	3025
16	186	2979	75	3042	111	2943
Total....	299	3004	122	3060	177	2966

the colored children born at term was 3004 grams—177 grams less than that in the whites. In the 122 blacks with normal pelvis the average weight was 3060 grams, while it was 2966 grams in the 177 patients with contracted pelvis—a difference of 94 grams.

Riggs,* in a report from this clinic, found the average weight of the children of 370 white and of 196 black primiparae of

all ages was 3224 and 2996 grams respectively—a difference of 228 grams. The figure usually given as the average weight of term white children is 3250 grams. A comparison of our findings with this figure, as well as with those of Riggs, shows that the children of young primiparae vary but little in weight from those born to older women. It is, therefore, evident that the relatively short labor of the young primipara cannot be explained on the ground that the baby is smaller than at a more advanced maternal age.

Maternal Mortality.—In our series of 500 patients there were four maternal deaths, a percentage of 0.8. All of these deaths occurred in colored girls. One died from eclampsia

TABLE 13
FETAL MORTALITY

	Toxemia	Syphilis	Cause not determined	Asphyxia	Destructive operations	Injected cord	Pneumonia	Total
White	3	2	4	2	0	1	1	13
Black	3	4	4	1	4	1	1	18
Total	6	6	8	3	4	2	2	31

on the third day after the the spontaneous delivery of a term child. The other three patients were delivered of premature children, one by manual dilatation of the cervix and version and extraction because of prolonged labor and intrapartum infection. She died suddenly two hours later, with a clinical diagnosis of hemorrhage and shock, but the findings at autopsy were negative. Of the other patients one died on the fifth day of the puerperium from streptococcus peritonitis and the other on the tenth day from pyelonephritis which had existed for weeks before delivery.

Fetal Mortality.—Of the 442 deliveries at term, 31 children were stillborn or died within the first two weeks of life, a percentage of 7.0. The causes of death are shown in Table 13.

CONCLUSIONS

Based upon the study of the 500 patients comprised in this report, it seems permissible to conclude that pregnancy and labor are attended by no greater danger in the young primipara than in the older woman. On the other hand, the duration of labor is actually shorter. As our figures show that the size of the children is not inferior to that noted in older women, and that abnormal pelves occur quite as frequently, this result must be attributed to the greater elasticity of the soft parts. Consequently, speaking from a purely obstetrical point of view, the ages under consideration appear to be the optimum time for the occurrence of the first labor.

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THE PROTECTIVE POWER OF SERUM IN PERNICIOUS ANEMIA AND OTHER CONDITIONS AGAINST HEMOLYSIS BY SAPONIN AND BY SODIUM OLEATE

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It is a well-known fact that human serum has the power to protect against hemolysis by many different hemolytic agents. In studies of the protective power of human serum against hemolysis by one of them, sodium oleate, it was found that this antihemolytic property was diminished in the serum from patients suffering from hemolytic anemia and from those in whom involvement of the liver and spleen in the disease process was a prominent feature.¹ The experiments establishing this point were undertaken with the idea that hemolysis in the hemolytic anemias might be due to a diminution in protective power of serum against a hemolysin normally circulating in the blood. For this reason sodium oleate was used

as the hemolytic agent because it is a substance which may readily be supposed to be present in the body under normal conditions. In view of these findings with sodium oleate it was thought wise to study also the protective power of serum against a hemolytic agent not present in the body and to compare the results with those obtained with sodium oleate. Saponin is a hemolysin against which serum has marked protective power and one which has not been demonstrated in the body. Accordingly, the experiments to be reported below were a study of the protective power of serum against saponin, a hemolytic agent not present in the body, and sodium oleate, one which probably is present normally.

Several observations of interest in relation to the hemolytic power of saponin and its inhibition by various substances have been reported. Wacker² noted that extracts from the stomach and intestinal mucosa inhibited the hemolytic action of saponin, and Frei³ that a similar action was exerted by hemoglobin solution. In his experiments Frei used hemoglobin from dog's corpuscles. Noguchi⁴ added the dyes eosin and erythrosin to saponin and found that by this treatment its hemolytic power was diminished. He also reported⁵ that, if red blood corpuscles were exposed to cobra-venom beforehand, their resistance to hemolysis by saponin was increased. Arrhenius⁶ recorded a similar increase in resistance to hemolysis of red blood cells that had been exposed to lecithin and to sodium oleate. Heuberger and Stepp⁷ tested the resistance to hemolysis by saponin of washed red blood cells from patients suffering from many diseases; the tables recorded show considerable variability of resistance in the different conditions studied. The least resistant cells were those from cases of pernicious anemia, and among the next resistant were those from patients with severe anemias. May⁸ expressed the opinion that variations in the resistance of red blood cells to hemolysis by saponin depended upon modifications in the relative proportions of lecithin and cholesterol in the cell. This investigator found that in pulmonary tuberculosis and typhoid fever the resistance of the red blood cells was diminished; and in icterus, in various types of nephritis, and in arteriosclerosis the resistance might be either increased or diminished. In pernicious anemia and in cancer showing a blood picture somewhat resembling that of pernicious anemia, May reported the resistance of the red blood cells to hemolysis by saponin to be the same as that from normal people. He concluded, therefore, that anemias were not due to increased fragility of the red blood cells. Ransom⁹ showed that not only in serum, but in the stroma of the red blood cells also, there was a substance capable of inhibiting hemolysis by saponin and identified this substance definitely as cholesterol. In this same report Ransom stated that cholesterol protected effectively against saponin hemolysis, and that lecithin did not. This latter observation is not in accord with the findings of Clark and Evans¹⁰ who reported that lecithin exerted some protective power against hemolysis by saponin, although it was much less potent in this regard than cholesterol. Of more immediate interest in relation to the points under investigation in this study are the findings of Port.¹¹ He measured quantitatively the protective power of human serum against hemolysis of human red blood cells by saponin and reported that the extent of this antihemolytic power of the serum was remarkably constant in normal persons and in patients suffering from a wide range of diseases. It was found to be increased in the lipemia of diabetes, in different kinds of nephritis, in lues and in typhoid fever. In three cases of pernicious anemia tested, however, the antihemolytic power of the serum showed a marked diminution; and to be compared with this is the normal antihemolytic value found in the serum from a case with a severe secondary anemia showing 1,000,000 red blood cells. The findings reported by Abderhalden and Weil,¹²

who tested the antihemolytic property against saponin of horse serum, are also of interest. They found a slight diminution in protective power in the serum from horses suffering from glanders and septic anemia, and a severe diminution in the serum of all horses having so-called "pernicious anemia." No other disease was found in which the reduction in antihemolytic power of the serum against saponin was great enough to compare with that in "pernicious anemia" and it was suggested, therefore, that this finding could be used as a diagnostic test.

In the investigations reported in this paper quantitative measurements were made of the protective power of human serum against hemolysis of guinea-pig corpuscles by saponin. The saponin used was a chemically pure specimen furnished by the Heyl Laboratories of Chicago, and taken just before use from the same 10-ounce bottle in which it was delivered. At the same time quantitative measurements of the antihemolytic property of the serum against sodium oleate were made. The technique employed and control experiments carried out were the same as already reported¹ in studies of the protective power of serum against hemolysis by sodium oleate, except that the dilutions of saponin were not so great as those for sodium oleate. A number of tubes, each containing 2 c. c. of a different dilution of hemolytic agent were set up in series and to each 0.25 c. c. of the serum to be tested for protective power against hemolysis was added. This was heated at 37° C. in the water-bath for half an hour, cooled for half an hour, and then 0.25 c. c. of a 0.75 per cent suspension of washed guinea-pig cells was added as indicator. This preparation was incubated at 37° C. in the water-bath for two hours, allowed to sediment in the ice-box for one hour, and then a reading of hemolysis was made. In the experiments with sodium oleate the tubes carried dilutions of the hemolytic agent of 1-45,000 to 1-100,000 in steps of 5000: *i. e.*, 1-45,000, 1-50,000, 1-55,000, 1-60,000, etc. In such a series normal serum regularly protected guinea-pig cells against hemolysis up to strengths of 1-45,000 to 1-55,000 of sodium oleate, so that any hemolysis in the tubes containing solutions of sodium oleate weaker than 1-55,000 might be considered as indicating a diminution in the protective power of the serum being tested against hemolysis by sodium oleate. Preliminary experiments with saponin, however, indicated that to obtain comparative results different dilutions of this hemolytic agent must be employed. For example, when the hemolytic power of saponin was tested with unprotected guinea-pig cells the following results were obtained:

Highest dilution showing	Some hemolysis	Complete hemolysis
Guinea-pig 1	1-140,000	1-40,000
Guinea-pig 2	1-140,000	1-40,000
Guinea-pig 3	1-160,000	1-40,000

Earlier experiments with sodium oleate, in which the same technique was employed, showed:

Highest dilution showing	Some hemolysis	Complete hemolysis
Guinea-pig 1	1-170,000	1-110,000
Guinea-pig 2	1-140,000	1-110,000

From these findings one may conclude that saponin is a weaker hemolytic agent than sodium oleate, for although the highest dilutions showing some hemolysis were essentially the same with both, complete hemolysis occurred with much weaker solutions of sodium oleate than of saponin. This difference in hemolytic power of sodium oleate and saponin was also pronounced in the preparations in which the guinea-pig corpuscles were protected by serum as in the technique used regularly in titrating the antihemolytic power of serum. As already noted, normal serum protected against hemolysis of sodium oleate up to strengths of 1-45,000 to 1-55,000. With saponin, however, the highest dilutions in which hemolysis was seen in the presence of normal serum were 1-18,000, 1-20,000 and 1-22,000 (Table I).^{*} Accordingly, in the experiments with saponin, the tubes of each series contained dilutions of 1-18,000 to 1-32,000 in steps of 2000, and if any hemolysis was seen in tubes containing solutions of saponin weaker than 1-22,000 it was considered as indicating a diminution in the protective power of the serum being tested against hemolysis by this agent.

Using the technique outlined in a previous paper,¹ we tested the protective power of the serum from a number of patients against hemolysis by saponin and sodium oleate. The results appear in Tables I, II and III.

The figures recorded in Table I are those obtained with serum from normal persons. Under Case 16 of this table are the figures for several titrations made on the same person at different times. The constancy in the degree of protective power of normal serum from different persons and from the same person at different times against hemolysis by saponin is noteworthy, and corresponds in this regard with that found

^{*} These findings suggest the possibility that saponin is not only a weaker hemolytic agent than sodium oleate, but also that human serum may protect more strongly against saponin than against sodium oleate.

TABLE I

RESULTS OF THE TITRATION OF SERA FROM NORMAL PERSONS FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SAPONIN AND SODIUM OLEATE

Number	Date	Highest dilution of saponin showing hemolysis	Highest dilution of sodium oleate showing hemolysis
1	5- 5-20	20,000
2	5-17-20	20,000
3	5-21-20	20,000	55,000
4	5-26-20	20,000	50,000
5	6- 9-20	20,000	50,000
6	6-16-20	20,000
7	7- 7-20	20,000
8	7-17-20	18,000	50,000
9	7-24-30	20,000	55,000
10	12- 8-20	18,000
11	12-23-20	18,000	55,000
12	12-30-20	20,000
13	1- 7-21	22,000	50,000
14	1-12-21	22,000	55,000
15	1-14-21	20,000	45,000
16	1-19-21	20,000	55,000
..	1-22-21	18,000
..	1-26-21	20,000
..	1-31-21	20,000
..	2-12-21	20,000	55,000
..	2-16-21	20,000	55,000

TABLE II

RESULTS OF THE TITRATIONS OF SERA, FROM PATIENTS HAVING NEITHER PERNICIOUS ANEMIA NOR LIVER-SPLEEN SYNDROME, FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SAPONIN AND SODIUM OLEATE

No.	Name	R. B. C.	R. B. C.	Hb. %	W. B. C.	P. M. N. %	Highest dilution of saponin showing hemolysis	Highest dilution of sodium oleate showing hemolysis	Diagnosis and remarks
1	P. G.	1-26-21	2,464,000	35	5000	73	26,000	75,000	Secondary anemia. Bleeding duodenal ulcer.
2	E. B. P.	1-28-21	4,504,000	50	13,600	75	20,000	70,000	Chronic infectious arthritis.
3	N. D.	1-25-21	4,320,000	77	7780	52	26,000	70,000	Cerebral hemorrhage. Old mastoiditis.
4	E. R. B.	1-31-21	4,848,000	100	6080	80	20,000	55,000	Hysteria; paralysis of left side.
5	J. R. S.	1-29-21	5,604,000	85	6560	58	24,000	65,000	Pleurisy; probably tuberculosis.
6	T. S.	2-10-21	4,032,000	76	7960	57	22,000	70,000	Luetic aortitis.
7	S. M.	2- 5-21	5,664,000	100	7500	..	22,000	60,000	Cerebro-spinal lues.
8	H. B.	2- 7-21	4,842,000	78	8800	76	20,000	60,000	Arteriosclerosis and nephritis.
9	R. T.	2-15-21	4,072,000	63	2880	76	24,000	55,000	Empyema.
10	G. G.	2-15-21	2,928,000	42	7400	60	28,000	70,000	Sporotrichosis.

TABLE III

RESULTS OF THE TITRATIONS OF SERA FROM PATIENTS WITH PERNICIOUS ANEMIA OR LIVER-SPLEEN SYNDROME FOR PROTECTIVE POWER AGAINST HEMOLYSIS OF GUINEA-PIG CORPUSCLES BY SAPONIN AND SODIUM OLEATE

No.	Name	Date	R. B. C.	Hb. %	W. B. C.		P. M. N. %		Highest dilution of sodium oleate showing hemolysis	Highest dilution of sodium oleate showing hemolysis	Diagnosis and remarks
1	A. T. W.	5-21-20	2,352,000	48	12,200	..	36,000	95,000	Hemolytic icterus.
		2-9-21	3,400,000	65	13,000	..	32,000	80,000	
2	J. H. W.	1-19-21	2,028,000	34	3,360	47	28,000	90,000	Primary aplastic anemia.
3	A. C.	1-26-21	26,000	90,000	Cirrhosis of liver.
		2-4-21	2,722,000	50	4,200	76	30,000	95,000	
		2-12-21	22,000	80,000	
		2-19-21	3,372,000	69	2,700	..	32,000	100,000	
4	W. D.	1-28-21	1,896,000	29	5,250	91	28,000	90,000	Myeloid leukemia of skin.
		2-2-21	1,544,000	29	9,850	97	36,000	95,000	Figures in P. M. N. % column include a few myelocytes.
5	H. H. C.	7-3-20	1,092,000	40	3,640	..	30,000	65,000	Pernicious anemia. Has had repeated transfusions.
		7-11-20	1,336,000	36	2,650	58	36,000	80,000	
		7-18-20	2,300,000	42	2,440	..	30,000	85,000	
6	A. J. K.	7-3-20	30,000	70,000	Pernicious anemia.
		7-11-20	1,760,000	38	3,400	..	30,000	55,000	
		7-18-20	1,888,000	29	3,000	..	30,000	85,000	
7	W. A. J.	6-13-20	2,436,000	53	3,720	33	32,000	70,000	Pernicious anemia.
		7-3-20	3,400,000	78	4,900	45	30,000	65,000	
8	F. T. H.	5-23-20	614,000	16	32,000	46	28,000	65,000	Pernicious anemia.
		7-13-20	3,952,000	68	4,000	86	30,000	90,000	Acute infection.
9	W. H. P.	7-15-20	1,700,000	45	3,500	57	32,000	70,000	Pernicious anemia.
10	C. H.	7-15-20	1,700,000	45	3,500	57	32,000	70,000	Pernicious anemia.
11	J. J.	1-7-21	1,280,000	35	8,750	38	22,000	50,000	Pernicious anemia.
12	W. B.	1-15-21	2,256,000	56	4,440	57	22,000	70,000	Pernicious anemia.
		1-24-21	3,354,000	47	2,124	24	32,000	65,000	Transfusion 1-18-21.
		2-4-21	1,516,000	55	1,650	25	32,000	100,000	Transfusion 1-21-21, followed by a severe serum reaction.
		2-9-21	1,698,000	34	4,640	50	32,000	95,000	
13	F. K.	1-14-21	1,696,000	39	7,240	63	20,000	55,000	Pernicious anemia. Patient is in very good shape. Last seen 30 months ago and has been well since.
14	J. W. B.	1-22-21	32,000	100,000	Pernicious anemia. In aplastic stage.
15	D. B. L.	3-7-21	1,208,000	28	6,400	66	30,000	95,000	Pernicious anemia. There was also a positive Wassermann reaction which became negative after salvarsan therapy.
		3-15-21	1,000,000	22	6,080	58	38,000	70,000	
16	F. S.	1-26-21	992,000	29	3,800	63	26,000	70,000	Pernicious anemia.
		1-31-21	784,000	20	3,040	67	30,000	90,000	
		2-4-21	2,144,000	33	1,924	64	30,000	80,000	
		2-9-21	1,464,000	23	2,800	66	32,000	90,000	
		2-16-21	1,576,000	35	2,600	54	32,000	75,000	
17	E. M.	1-26-21	4,000,000	75	5,080	55	30,000	60,000	Pernicious anemia. Patient has been in good shape for years. Symptoms primarily of C. N. S.

against hemolysis by sodium oleate at this time and previously noted. It will be noticed, however, that a serum may show with one hemolysin the highest antihemolytic titer ever seen with normal serum, and the lower limit of normal with the other. This probably means minor variations in the hemolytic power of the hemolytic agents as made up from day to day. On the other hand, it may mean slight variations in the antihemolytic power of normal serum; and if so, it suggests that this property of serum is due to a different substance in it for each hemolysin, and that these vary independently of each other. No definite statement may be made, but it appears likely that both of these factors are operative in this connection.

The figures recorded in Table II are those obtained with the sera from patients having either no anemia, a slight anemia, or a severe anemia associated with various diseases. All patients in this list, except No. 4, were very ill at the time the serum was tested. None had pernicious anemia or any evidence of involvement of the liver or spleen in the disease process present. The degree of protective power against saponin shown by the serum varied somewhat in these patients, and some reduction was common, although in the cases tested it was not severe. The degree of antihemolytic power was diminished against either sodium oleate or saponin in all except one of these sera. It was not, however, the same at all times for both hemolysins. Four cases showed normal protective power against hemolysis by saponin and some diminution against hemolysis by sodium oleate; and one case showed slight diminution in protective power against hemolysis by saponin and normal antihemolytic power against sodium oleate. The sera of five of these patients showed a normal protective power against hemolysis by saponin; and with sodium oleate two had a normal antihemolytic power and three had only a minimal degree of diminution. So, although the serum of a patient may not show the same degree of antihemolytic power for both sodium oleate and saponin, in general it may be said for saponin, as already reported for sodium oleate, that a diminution of antihemolytic power may or may not be present in this class of cases; and that, when present, it is not severe.

The figures recorded in Table III are those obtained with the sera from patients with the type of disease in which a marked diminution in protective power against hemolysis by sodium oleate had been previously found, namely, those suffering from hemolytic anemia or conditions in which changes in the spleen or liver are prominent features. In the sera from these patients the degree of antihemolytic power for saponin varied markedly among different individuals and also varied in the same individual at different times. A severe diminution in protective power against saponin was present in all but four of these cases, and was found at one time or another in every case tested more than once. Three of the four cases which showed normal antihemolytic power for saponin had normal values for sodium oleate also, and in the fourth case the diminution in protective power against hemolysis by sodium oleate was slight. All the cases which showed diminution in protective power against saponin had a diminution in antihemolytic power for sodium oleate. In many instances the degree of diminution in protective power against the two hemolytic agents was not the same; but when there was a severe reduction for one hemolytic agent, there was always some reduction for the other, usually quite pronounced.

When the antihemolytic values of the sera from the cases presented in Table II are compared with those in Table III, marked differences are apparent. The serum from none of the cases in Table II permitted hemolysis by saponin in dilutions greater than 1 to 28,000, or by sodium oleate in dilutions greater than 1 to 75,000. Among the 17 cases in Table III, however, 10 showed hemolysis by saponin in dilutions greater than 1 to 28,000, and 11 hemolysis by sodium oleate in dilu-

tions higher than 1 to 75,000. The averages of figures obtained in titration for protective power of serum found in the three classes of patients appear in Table IV. In Table V the results

TABLE IV

AVERAGES OF THE TITRATION VALUES OF ANTIHEMOLYTIC POWER FOR SAPONIN AND SODIUM OLEATE OF SERA FROM: (1) NORMAL PERSONS; (2) PERSONS WITH HEMOLYTIC ANEMIA OR LIVER-SPLEEN SYNDROME; (3) OTHER PATIENTS TESTED

	Number of titrations	Highest dilution of saponin showing hemolysis	Highest dilution of sodium oleate showing hemolysis
Normal persons.....	21	19,800	52,500
Patients with hemolytic anemia or spleen-liver syndrome.....	35	27,085	80,000
Other patients.....	10	23,200	65,000

TABLE V

TABULATION OF THE SERA FROM THREE CLASSES OF PATIENTS: (1) NORMAL PERSONS; (2) PATIENTS WITH HEMOLYTIC ANEMIA OR LIVER-SPLEEN SYNDROME; (3) OTHER PATIENTS TESTED; ACCORDING TO THEIR ANTIHEMOLYTIC POWER FOR: (A) SAPONIN; (B) SODIUM OLEATE

A—SAPONIN

Dilutions of saponin	15,000	20,000	22,000	24,000	26,000	28,000	30,000	32,000	Total
Normal persons.....	4	15	2						21
Hemolytic anemia or spleen-liver syndrome.....	6	4		5	4	7	9		35
Other patients.....	3	12	12	12	1				10

B—SODIUM OLEATE

Dilutions of sodium oleate	45,000	50,000	55,000	60,000	65,000	70,000	75,000	80,000	85,000	90,000	95,000	100,000	Total
Normal persons.....	1	4	7										12
Hemolytic anemia or spleen-liver syndrome.....	1	2	1	3	6	1	4	3	6	5	3		35
Other patients.....		2	2	1	4	1							10

of all the titrations appear in summary. From these tables it is seen that the type of patient in which the serum shows the most severe reduction in antihemolytic power for saponin is the same as that in which the most severe reduction in antihemolytic power for sodium oleate is encountered.

The variations in antihemolytic power for saponin of sera from patients with hemolytic anemia did not run parallel with changes in the blood picture. It was noteworthy, however, that regardless of the degree of anemia present when the titration was done, when the antihemolytic power of the serum was high, the patient regularly said he was feeling better, and when a low antihemolytic titer was found, the patient complained of feeling badly. This same observation

is true for variation in the antihemolytic power of serum from these patients for sodium oleate, and was made also in our earlier study when this hemolysis was used.

In summary, the following facts have been demonstrated in these experiments in which quantitative measurements were made of the protective power of human serum against hemolysis of guinea-pig cells by saponin and sodium oleate.

A. SAPONIN

1. The protective power of serum from normal persons against hemolysis by saponin is remarkably constant in degree.

2. In many diseases with anemias of various grades and types the protective power of the serum against hemolysis by saponin is diminished.

3. The decrease in protective power against hemolysis by saponin is most marked in anemias which are hemolytic in character and in other conditions in which involvement of the liver and spleen is a prominent feature in the disease process. In this class of cases, the diminution is very striking, both in degree and the regularity with which it is found. It varies markedly and seems to parallel more closely the general condition of the patient than the blood picture at the time the titration is done.

B. SODIUM OLEATE

1. When the protective power of human serum against hemolysis by sodium oleate is measured quantitatively, variations are found as when saponin is used as the hemolytic agent. Although the degree of antihemolytic power of one serum is not always exactly the same for both hemolytic agents, in general it may be said, in regard to types of patients, that the variations found with sodium oleate are the same as those with saponin.

CONCLUSIONS

The sera of patients with hemolytic anemia and other conditions in which the liver and spleen are prominently involved in the disease process, as compared with the sera of normal persons and of patients not suffering with either of these maladies, show a marked diminution in protective power against hemolysis by saponin, and by sodium oleate. Saponin is a hemolytic agent foreign to the body, and sodium oleate is one that is probably present in the body normally.

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THE ESTABLISHMENT OF COLLATERAL CIRCULATION FOLLOWING LIGATION OF THE THORACIC DUCT

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The general physiology of the lymphatic system has been studied from various angles. The discovery of the lymphatic system by Asellius¹ in 1627 was due to an observation correlating digestion with the activity of lymphatic vessels. Clinical pathological findings stimulated individuals to wound the thoracic duct in the chest and study the subsequent phenomena (Lower²); while others (Flandrin,³ Colin⁴) ligated the thoracic duct in the neck and observed the influence of this procedure on the animal economy. Later when the knowledge of chemistry became advanced, thoracic duct fistulae were made with a view toward studying the fluid in the duct under ordinary conditions and as modified by the digestion of various foods, *e. g.*, fats. Again, through the work of Heidenhain⁵ and Starling,⁶ the effects of lymphagogues of various classes were investigated. Finally, the thoracic duct was studied in regard to its power of supplying blood cells, chiefly small lymphocytes, to the blood stream (Biedl and v. Decastello⁷).

In this report an attempt has been made to review the literature of the experimental ligation of the thoracic duct, and also to give a description of a new method for ligating that vessel.

REVIEW OF LITERATURE

Probably the first one to attempt the ligation of the thoracic duct was Duverney⁸ in 1675. He ligated the subclavian vein near the entrance of the duct, in some cases applying the ligature proximally and in others distally to the entrance. The dogs lived for a fortnight.

Sixteen years later in 1691, Flandrin,⁹ a well-known veterinarian of his time, chose to repeat the work of Duverney, but preferred the horse, since he considered the lymph vessels in the smaller animals as too inconstant in their location. In the first experiment the animal died at the end of three days; subsequent dissection showed nothing of particular interest. In the second case a young horse was used. It experienced no ill effects immediately following the operation, nor in the subsequent 15 days during which it was allowed to live. "Perfect suppuration" took place in the wound, and with the possible exception of a distension of the thoracic duct at its point of ligation, the autopsy revealed no marked difference

in the appearance of the lymphatics. Similar results were obtained with 10 more horses; the majority of the animals were killed 15 days following ligation, and the only abnormal finding was a distension of the thoracic duct at its point of ligation, with occasional extension of this distension down into the chest. One animal was kept for two and a half months after operation, and at autopsy showed a cicatrized thoracic duct. It is also interesting to note that Flandrin was among the first to introduce a cannula into the thoracic duct for the purpose of studying the properties of the lymph. He concluded that the lymph reaches the blood-stream through other channels than the thoracic duct.

In 1798 Sir Astley Cooper¹⁰ reported the results of some experimental work involving the ligation of the thoracic duct in dogs. He was stimulated to undertake the experiments because he had observed three cases of obstruction to the thoracic duct in man. In two of these cases he found evidence of collateral circulation; in the third case no such evidence was found. In the first experiment the dog died 48 hours after the duct had been tied; dissection showed the viscera obscured by an effusion of chyle; particularly was this the case with the pancreas and the kidney; a small quantity of chyle had extravasated into the peritoneal cavity; the lacteals were empty; the thoracic duct was twice its normal size. These findings Cooper explained on the basis of a ruptured cisterna chyli. The second experiment was a great deal like the first, only the dog lived 2 days longer. The autopsy findings were the same; the cisterna chyli had burst and the extravasation had hidden entirely the aorta and the vena cava. In a third experiment the thoracic duct was cut and allowed to drain into the tissues; the animal died on the fourth day; dissection showed a pouch of lymph in the wound. These experiments were repeated always with the same results; no animal survived the tenth day. In one case Cooper noticed that a branch of the thoracic duct went over to the right trunk; also that the amount of extravasation bore a direct relation to the amount of food which the animal had just previously taken. Cooper did not give any evidence, *e. g.*, by injections, to support his belief that the cisterna had ruptured.

The only other investigator besides Cooper to report rupture of the cisterna chyli following ligation was Mayer.¹⁰ Contrariwise, Rogers¹¹ observed no ill effects from ligation.

In 1821, Magendie¹² reported the experiments which Dupuytren made on several horses. Dupuytren ligated the thoracic duct in the neck, and observed that some animals died at the end of five to six days; while others did not seem to be affected by the operation. Subsequent injections showed that in the animals that died there was a complete obstruction of the thoracic duct; whereas, in those that survived—and one case was allowed to go for six weeks—a collateral circulation was always demonstrable. Accordingly, it was argued that the death of those animals that lived only five to six days, and in which the duct was tied, was due to the deprivation of nourishment furnished via the thoracic duct; and the only reason that the other animals lived longer was because collaterals to the thoracic duct had formed and had thus transmitted the necessary food substances to the blood stream.

Leuret and Lassaigue¹³ tied the thoracic duct in the neck of a dog and found that the animal lived perfectly well, even gained weight. At the end of 58 days it was sacrificed at the height of digestion; dissection showed that the canal had been satisfactorily ligated. The only findings of importance were that the duct, the cisterna and the mesenteric lymphatics contained a small amount of chyle; and that the mesenteric veins seemed larger than normal. They believed that the chyle took an abnormal course to reach the blood, and that this path was from the cisterna chyli to the portal vein. They proved this assertion to their own satisfaction when they ligated the portal vein and found blood in the cisterna chyli and at the commencement of the thoracic duct. They also found that when the blood and chyle were taken from the thoracic duct and allowed to stand, two distinct clots were formed: the one, red, remained below; while the other, pink, evidently chylous, remained above.

Colin⁴ in 1873 reviewed briefly and analyzed to some extent the work of the above mentioned investigators and then related his own experiences with ligations of the thoracic duct in various animals. All ligations seem to have been done in the neck. His first experiment was on a bull. The animal made an uneventful recovery and was sacrificed at the end of six or seven weeks when it was found that the thoracic duct was obliterated to the extent of five or six centimeters below the point of ligation, but that collaterals had formed at this point and had extended to the regular entrance of the thoracic duct into the veins. He repeated these experiments on two cows, taking care to include in his ligations all branches of the thoracic duct, and found at the end of 24 hours that there was a marked distension of the lymphatic system, as well as an extensive extravasation into the mesentery. He believed that the animals would have died from the operation if they had been kept long enough. On a bull 18 months old ligation resulted in death at the end of nine days; lymph did not reach the veins. After the completion of these observations on these large animals he made use of dogs for further experiments. One dog died from chylothorax on the day

following the operation. Two other dogs died at the end of 20 and 25 days respectively, having refused food for almost the entire time. In both cases the thoracic duct was thickened and obliterated at its upper end and injections did not reach the veins. These animals "died the death of starvation." Colin concluded that ligation of the thoracic duct was not always followed by the same results. In those cases in which the duct was double, or anastomosed with the right thoracic duct, lymph reached the blood stream, and the animal recovered; whereas, if the duct were single and when ligation had been complete, death resulted promptly. He called the attention of future investigators to the fact that the entrance of the thoracic duct into the veins is so variable that in some cases it is absolutely impossible to prevent the lymph and chyle from reaching the blood stream.

However, Schmidt-Mühlheim¹⁴ devised an operation which seemed to take care of all possible variations of the entrance of the thoracic duct into the veins. The problem which Ludwig gave Schmidt-Mühlheim was to establish whether the digestion products of albumen reached the blood stream via the thoracic duct or not. From this it can be readily seen what an exaggerated importance physiologists up to that time attached to the thoracic duct. He used dogs for his experiments and operated without an anæsthetic or curare. He must be credited, however, with using strict aseptic technique during the operation, and, accordingly, ranks among the first to observe aseptic measures on experimental animals in the solution of biochemical problems. The operation consisted in isolating and tying off successively, on the right side as well as on the left, the v. jugularis externa, v. jugularis interna, v. axillaris and v. anonyma. Also the two thoracic ducts, right and left, were cut between ligatures. The operation did not affect the general condition of the animals in any particular; but since they were sacrificed at the end of six or seven days, no report of the effect of long continued ligation of the thoracic duct on the health of the animal is available. The complete occlusion of the entrance of the thoracic duct into the veins of the neck was demonstrated immediately after death by the injection of Berlin blue into the cisterna chyli at a pressure of 40-50 mm. of mercury; this was followed by dissection of the duct to the point of ligation. Again, the superior vena cava was also opened and a search made for evidence of the dye. Only those cases in which complete obstruction had been established were considered satisfactory; and following careful chemical analysis Schmidt-Mühlheim concluded that in complete obstruction to the chyle-flow into the blood vascular system, the digestion and absorption of albumens, as well as their conversion into urinary products, are the same as when the chyle is unobstructed in its passage to the blood stream. This result was an advance in the field of physiological chemistry.

The autopsy findings in these experimental dogs differed particularly in one essential respect from the observations of Cooper.⁸ Cooper, it will be remembered, found rupture of the cisterna chyli following ligation with extensive extravasations; however, he failed to verify the rupture by injections.

Schmidt-Mühlheim, on the other hand, convinced himself by injecting directly into the cisterna with a solution of Berlin blue at a pressure of 40-50 mm. of mercury, and keeping this pressure up for hours that, in spite of wide extravasation, the cisterna chyli was intact; in no case was any dye found in the perivascular tissue. Marked distension of the lymphatics, chiefly abdominal, as well as enlargement of the lymph glands, was observed constantly. Also, it was noted, that a retrograde injection of the large lymphatic trunks emptying into the cisterna was possible to the extent of 3-4 cm., because the valves had become incompetent; particularly was this true of lymphatics coming from the liver. On the contrary, a retrograde injection of smaller lymphatics was never possible. He added that the mucosa of the intestine always had a normal appearance.

In 1883 Boegehold¹² reviewed the important work done on the thoracic duct. He reviewed not only the anatomy with its numerous variations, the physiology, the pathology, and clinical features involving the thoracic duct, but also gave an account of the experimental work done on the thoracic duct, adding the results of some of his own experiments. He was interested in studying the effects of wounds of the thoracic duct, and to that end cut the thoracic duct partially or entirely in the chest of dogs. He found that cutting about one-quarter of the periphery of the duct led to fibrin formation and clotting at the damaged area; cutting completely across the duct caused chylothorax and death; wounding the duct slightly, after it had been tied off effectually in the neck according to the method of Schmidt-Mühlheim,¹³ resulted at the end of three days in a small amount of fibrin formation at the point of injury. Accordingly, he argued that absolute integrity of the thoracic duct was not essential to life. This was shown also by a number of clinical cases that suffered no apparent ill effects from complete obstruction to the duct. Also it seemed as if collaterals were rapidly developed subsequent to obstruction; but no proof of this was given.

A new departure in ligation of the thoracic duct was effected in 1898 by Stüler.¹⁴ This author, after referring in uncomplimentary terms to the hypothesis of v. Schwerdt¹⁵ regarding the pathology of Morbus basedowii with respect to the formation of subcutaneous collateral lymphatics, gave the results of his experimental work on the ligation of the duct in rabbits. Under aseptic precautions and ether anesthesia he ligated the thoracic duct high in the abdominal cavity, just below the diaphragm. Four experiments in all were made. In the first case the aorta was damaged and the animal bled to death; in the second case the diaphragm was ruptured at operation and the animal later died of peritonitis. The fourth animal also died of peritonitis. The third animal, however, survived and gained weight. A piece of the supposed thoracic duct removed at the time of operation showed microscopically that it actually belonged to the thoracic duct. The author intended to report later the findings in this animal, but no subsequent record is available.

Lucibelli¹⁶ published the results of his work on the effects of ligation of the thoracic duct. He used two large dogs as

his subjects and gave them milk four or five hours before the operation as an aid in identifying the duct. With chloroform or Ricet's solution as anesthetics and with sterile precautions, the operation was performed in the left side of the neck. In the first dog the operative wound became infected and continued to discharge pus for from 20 to 25 days after operation. At the end of this time the dog ceased losing weight and began to gain; his general condition also improved. At the end of two months from the time of operation the animal was in fair condition and the various examinations of the different body fluids were made. These examinations were very elaborate and included a complete urine analysis, isotonicity of the blood, red, white and differential blood counts. As a control for these findings an entirely different dog of the same weight, but living under the same conditions, was used. The first dog gradually became worse and died 3 months and 11 days after the operation. Autopsy showed a spleen five times normal size, mesenteric glands hypertrophied, glands of neck large, large heart, liver and pancreas. However, no injections were made to see whether the ligation in the neck was complete. The second dog did not have any post-operative infection. At the end of three months the same extensive examinations of the urine and blood were made, and immediately thereafter the dog was killed. Autopsy showed no loss of weight as in the previous case; slight engorgement of lymph glands; spleen four times normal size; small amount of fluid in pleural cavities; nothing unusual otherwise was observed. There also followed an exhaustive description of the histological findings of the tissues removed from the two animals. From these data, Lucibelli concluded that complete ligation of the thoracic duct caused death in the dog; that partial ligation caused little disturbance because collateral lymphatics established themselves soon; that complete ligation caused changes in all the organs and impaired their secretory function; that changes in the histological picture were due to the toxicity of stagnant lymph; and that of various causes for oedema, change in the lymphatics was one.

The most recent work on the lymphatic system that involved ligation of the thoracic duct, is that by Bunting and Huston.¹⁷ These authors, in considering the fate of the lymphocyte, resorted to ligation of the left thoracic duct and jugular vein just before the duct entered, and in some experiments the neck lymphatic trunks on the right side were also tied. All their work was done on the rabbit. They found that this procedure, coupled with splenectomy, produced a marked but temporary decrease in the absolute number of lymphocytes in the circulating blood. They also noted the numerous anomalies of the thoracic duct, and in one case observed anastomosing lymphatic vessels leading through the thymus to the right side.

In the foregoing brief review of the literature regarding the ligation of the thoracic duct as it affects the general economy of the animal, it can be seen that a gradual change in the opinion concerning the importance of the thoracic duct has taken place. At first it was held that ligation of the duct was fatal because the necessary alimentary juices no longer

reached the blood stream (Cooper⁹). Then it was argued that only those cases in which the ligation was complete were fatal; whereas, when all the branches of the thoracic duct were not included in the ligation and chyle could thus get into the blood stream, the animal survived (Magendie¹¹). Finally, it was believed, even complete ligation of the duct had no evil effect on the animal (Leuret and Lassaigne,¹² Schmidt-Mühlheim¹³).

DISCUSSION OF LITERATURE

In considering the literature quoted above, it is evident that no conclusive work has been done on the question regarding the effects on the animal of complete ligation of the thoracic duct and its branches, as well as noting the effects of such a ligation after a comparatively long period of time. Duverney⁸ obviously accomplished nothing with the ligations of the subclavian vein either proximal or distal to the entrance of the thoracic duct. Flandrin⁵ anticipated Leuret and Lassaigne¹² by 30 years; yet he gave no evidence to support the complete ligation of the duct. Sir Astley Cooper⁹ and Mayer¹⁰ claimed that ligation of the thoracic duct is lethal. Cooper claimed that rupture of the cisterna chyli accounted for the extreme extravasations; however, Schmidt-Mühlheim¹³ showed by injections that with similar extravasations the cisterna was not ruptured. Indeed Cooper did not describe the rupture to any extent, but considered it a matter of fact. Magendie¹¹ believed that death of the animal at the end of 5 or 6 days was due to the effects of the ligation and nothing else. In all probability these deaths were due to infection. Again, Magendie used injections to trace out the lymphatic paths, and in this respect his work was better than that of many investigators who worked a hundred years later. Leuret and Lassaigne¹² on the basis of one experiment found that ligation had no evil consequences. However, they operated only on one side of the neck, satisfied themselves by dissection without the aid of previous injection that the duct really was tied off; postulated that the chyle took a different course, namely, from the cisterna chyli to the portal vein, and proved this again to their own satisfaction, by simply ligating the portal vein and finding, as a result, some blood in the cisterna chyli. Colin⁴ after numerous experiments on various domestic animals came to the same conclusion as Magendie¹¹ 50 years before. In his first animal he obviously did not get all the branches of the duct in the neck successfully ligated. In the case of the two dogs that lived respectively 20 and 25 days after operation, infection was also probably the cause of death, since the animals refused all food and behaved so differently from other dogs in which the duct was really tied without infection. In this respect the careful work of Schmidt-Mühlheim¹³ is noteworthy. Up to this time all operative work had been done without antiseptic or aseptic precautions, and although "perfect suppuration" took place, nevertheless, this very fact vitiated practically the entire experiment. Schmidt-Mühlheim made the first serious attempt to prevent the chyle from reaching the blood stream; and furthermore, he used injections to make sure that the ligations were complete. It would have

been interesting to know what would have taken place if the animals had been kept longer than seven days. Unfortunately, all the animals were sacrificed within a week from the time of operation. However, to tie off practically the entire superior vena cava is a considerable operation and a great shock to the animal; nevertheless, the animals recovered from the operation very satisfactorily. Even this operation would not necessarily always shut off the chyle from the blood stream, since it is possible that branches of the thoracic duct could enter the axillary vein distally to the point of ligation and eventually reach the heart through the collateral venous circulation which obviously would be established. The advantages of this operative procedure were at once recognized and frequently used by biochemists (Munk and Friedenthal²⁰). Boegehold¹⁸ also stated that ligation of the thoracic duct exerted no deleterious effect on the animal, basing his argument on clinical cases as well as on the result of his last experiment, in which he ligated the thoracic ducts according to the method of Schmidt-Mühlheim. In this animal he made a small injury to the duct, and three days after this found a small clot at the site of injury. Here the same objections can be raised as were advanced with Schmidt-Mühlheim; besides this, injection of the lymphatics was not made to ascertain whether the ligation was complete. The experiments of Stüler¹⁹ are as novel as they are inconclusive. All that can be gathered from his work is that a complete ligation of the thoracic duct was attempted in the abdomen; that a large lymphatic trunk, probably the thoracic duct, was ligated; that the animal recovered and gained weight; and that the subsequent findings in the animal were promised but are not available. Lucibelli¹⁷ tried to determine what effects ligation of the duct would have on the animal economy, particularly when studied several months after the operation. His animals were killed at the end of three months, which is the longest time any animals with ligated thoracic ducts were ever allowed to survive. Unfortunately, his work is not without serious objections. In the first place he ligated the duct on one side of the neck only, although previous work, particularly that of Schmidt-Mühlheim,¹³ had shown how frequent it was that branches of the thoracic duct went to the right side, and how essential it was to ligate not only the thoracic duct on one side, but also to ligate the large veins in the base of the neck on both sides, as well as to ligate the right thoracic duct. That the dog is no exception in having many anomalies in the entrance of the thoracic duct into the veins was previously emphasized by Kufferath.²¹ In the second place, one of the two animals had a postoperative infection lasting nearly a month. This infection alone discounted the value of the results obtained by careful chemical analysis of the urine and blood. Again, he used as controls, not the same animal before operation, but preferred an entirely different dog of about the same weight as the experimental animal and kept under the same conditions. Also, judging from the urinary findings, the first animal had what Lucibelli called a chronic interstitial nephritis. Accordingly, no animal with a kidney lesion, even though the thoracic duct be securely tied, could be compared

with a normal animal, particularly in so far as the urinary findings are concerned. The presence of the infection vitiated also all conclusions regarding the histological changes purported to be consequent to a thoracic duct ligation. There is, furthermore, no evidence supported by post-mortem procedures to substantiate the conviction that the ligations were successful; and the absence of confirmatory examination alone makes the whole work faulty. It is needless to discuss the author's contention that his two experiments support the view that oedema is due to stagnant lymph formation. Finally, in the work of Bunting and Huston¹⁹ there was no evidence to support the belief that the thoracic duct was completely ligated. Their work did show that the rabbit is also no exception to the general rule which holds that the entrance of the thoracic duct into the veins of the neck may be extremely varied.

Before proceeding to the experimental work, it may be added that numerous investigators (Kunkel,²⁰ Fleischl,²¹ Kufferath,²² Harley,²³ Josué,²⁴ Wertheimer and Lepage,²⁵ Davis and Carlson²⁶) also ligated the thoracic duct and studied the effect of simultaneous ligation of the biliary duct, or subsequent changes in the number of lymphocytes in the blood. None of these authors operated in a way to preclude all possibility of anomalous collateral circulation, nor did all inject their specimens post-mortem to be sure of the course of the lymph.

The Russian literature also contains reports of work done on the ligation of the thoracic duct. Some of the work is experimental (Khlopin²⁷), in which the absorption of fats was studied; others (Vitlin²⁸), reported a case of injury to the duct, and reviewed the literature on other cases of injury to the duct. It is interesting to note that Vitlin²⁸ and Temkin²⁹ took the same case from the clinic of Professor Rotter in Berlin as a stimulus for their respective articles.

At the suggestion of Dr. Cunningham, and with his frequent advice, experiments were undertaken to determine what effect on the general economy of the animal ligation of the thoracic duct would have; also what collateral circulation, if any, would be established. It is a pleasure to thank Dr. Sabin and Dr. Cunningham for their interest and help during this investigation.

EXPERIMENTAL RESULTS

In all the experimental work the cat was used exclusively, chiefly because it is a convenient and very satisfactory laboratory animal, and also because the lymphatics of the cat have been described to a considerable extent. The animals were for the most part young adult male cats, seemingly in good health. Food was withheld for 24 hours before the operation which was performed under ether anesthesia and with strict aseptic precautions. During the operation, the animal received the benefit of hot-water bottles and after operation it was placed in a warm cage. All the animals were weighed immediately before the operation and at various times after the operation.

Operative Procedures.—At first, attempts were made to ligate the thoracic duct in the neck. In these cases the animals were fed cream five hours before operation in order to make

the duct more conspicuous; at times this advantage was heightened by adding Scharlach R to the cream. Several of these operations were successful in so far as injections from below showed that the duct had been effectively ligated. But it was soon evident that this procedure did not produce enough positive results to make it reliable for work which required absolute ligation of the duct, for frequently the duct had numerous entrances into the veins of the neck, and it was not possible to operate without feeling doubtful about the ligation of all the branches.

Accordingly it was decided to try to ligate the duct high up in the chest, before all the anomalous branches were given off. Because of the consequent pneumothorax following opening of the chest, intratracheal ether was given. A calibrated No. 13 F soft rubber catheter with accessory openings at its end was inserted into the trachea. Air for the apparatus was supplied by a foot bellows which was connected to a mercury safety valve adjusted to 20 mm. of mercury, and led over ether in a Woulfe bottle, which was further provided with a by-pass direct to the catheter. With this apparatus all gradations from pure air to heavily saturated ether vapor could be administered at physiological pressures. The operation consisted in going into the second or third intercostal space, spreading the ribs apart, isolating the carotid artery and vagus nerve, inclosing as much of the surrounding tissue as possible in a ligature, and closing the cavity. This operation also was not successful, because isolation of the vagus nerve and carotid artery was often difficult, and besides branches of the duct to the right side were completely missed by this undertaking.

The Operation.—Finally it was decided to operate lower down in the chest, and this procedure gave the desired results. The animal was placed on its right side, with the left front leg drawn a little upward and forward. The left side of the chest was shaved over an area extending from the third to the tenth intercostal spaces, and from the midline in the back to the parasternal line. Iodine technique to the skin was employed. A transverse incision about 4.5 cm. long was made over the sixth or seventh intercostal space, extending forward from a point about 4 cm. from the midline of the back. Dissection was carried down to the latissimus dorsi muscle, the fibers of which were split longitudinally. By palpation the desired intercostal space was determined and dissection was carried down through the serratus muscle to the superior border of the rib bounding the inferior portion of the intercostal space; this was done to avoid damaging the intercostal vessels which course superiorly. One was sure of good exposure when it was necessary to cut a few lateral fibers of the sacrospinal muscle. The intercostal muscles were then severed along the superior border of the rib, care being taken to cut to a uniform depth. After the pleura was reached, the tip of a pair of blunt-pointed scissors was thrust into the pleural cavity and the opening enlarged. A small pair of self-retaining rib retractors were inserted and the opening widened to about 3 cm. The lungs collapsed considerably and did not interfere with further work, particularly when the intratracheal pressure was kept low.

The thoracic duct was usually not seen because it contained no chyle. The adventitia over the anterior surface of the aorta was grasped with forceps and drawn slightly toward the opening. The adventitia inferior to the forceps was then carefully dissected from about the aorta as much as possible and an aneurysm needle with a fine silk thread was passed around the aorta; this thread served as a traction suture on the aorta. A right-angled aneurysm needle was then inserted from the left to the right side of the aorta and under it, and turned through an angle of 180° , so that the point rested on the right side of the vertebral column near the midline. The point was allowed to pass along the vertebral column to the left side until it appeared at the sympathetic chain. The tissues included in this ligature were the thoracic duct and sometimes the azygos vein. The aneurysm needle was then passed under the aorta from its right to its left side, the tissues of the previous ligature were again included, but chiefly was it desired to get the tissue between the vertebral column and the esophagus. Sometimes it was necessary to include more tissue about the esophagus in a third ligature; the ligation was thus completed. The ribs were approximated with a double medium silk suture; interrupted sutures for the muscle; the usual subcuticular stitch for closure. No dressing was applied but the iodinized area was carefully washed with ether to avoid any iodine irritation with consequent damage to the incisions. The entire operation from incision to closure did not take more than half an hour.

Post-Operative.—The animals made a satisfactory recovery following operation. They frequently ate on the day after operation. There was no evidence of diarrhoea, oedema or respiratory distress; slight abdominal tenderness was frequently observed. The animals were weighed at intervals following the ligation; the results were not uniform. Some of the animals gained weight, some lost, whereas the one which was sacrificed 66 days following the operation, and showed absolute ligation of the duct, evidenced little change in weight. On a series of animals leucocyte counts and differential blood counts were made to study chiefly the lymphocyte change following the operation. This work will be reported later.

The cats were sacrificed from 24 hours to 77 days after ligation. Under ether anesthesia the abdomen was opened and the mesenteric lymphatics of the small intestine were injected with a saturated aqueous solution of Berlin blue. The ordinary hypodermic syringe with a No. 28 needle was found to be preferable to larger Record syringes, since the pressure of injection could be regulated better. The chief point in injecting the mesenteric lymphatics was to avoid any possible injection of veins, for if the dye reached the blood stream other than through lymphatico-venous communications, the experiment would be inconclusive. Thus, if the subserous layer or muscular coats of the intestines were injected, then the dye could possibly enter the veins as well as the lymphatics. The same objection held for the mesenteric lymph gland, since a direct injection of a lymph gland would also entail the possibility of striking veins as well as lymphatics. During the injection of the last cubic centimeters of the dye,

the animal was killed, and the injection was continued until the ventricle stopped beating. This was done to insure a sufficient amount of dye at the lymphatico-venous communications. The blue pigment was usually seen in the lungs after the chest was opened and the course of the injection with respect to the thoracic duct studied. Frequently the animal died during the course of the injection from a pulmonary embolus caused by the injected material. Material for sections was cut from various organs; the remainder of the animal, except the head and extremities, was fixed in formalin and saved for further dissection.

TYPES OF COLLATERAL CIRCULATION FOUND AFTER LIGATION OF DUCT

As a result of these dissections, two general types of collateral circulation were established. The one type consisted of a collateral circulation to the right thoracic duct, the other type comprised those cases in which the lymph entered the azygos vein or its branches.

The first type is illustrated by Fig. 1 which shows the collateral circulation established to the right thoracic duct. It may thus be justly said that this case illustrates absolute ligation of the thoracic duct and the periaortic lymphatic plexus. The animal from which the figure was drawn was sacrificed one week after operation. The structures of interest were taken out *en bloc*, dehydrated and cleared in oil of wintergreen. Attention is called to the absolute ligation of all lymph drainage to the left side of the neck. Below the point of ligation is a small but significant lymphatic plexus supplied by a large branch coming off from the thoracic duct. From the superior aspect of this plexus a small branch is seen to course on the azygos vein to a small gland at the mouth of the subclavian vein, and from there two trunks finally join and empty into the junction of the jugulo-cephalic trunk and the internal jugular vein.

Attention is also called to the general appearance of the abdominal lymphatic vessels soon after ligation of the thoracic duct. An oedema developed about the large lymphatic vessels, particularly around the cisterna chyli and its large trunks. This oedema depended in its appearance on the nature of the fluid in the lymphatics. If these were laden with chyle, the oedema was chylous in nature; if no chyle were present, the perivascular tissues were bathed in a limpid, slightly yellow-tinted, clear fluid which seemed to contain small fatty droplets. In no case did this oedema resemble ascites to any degree. Likewise, the cisterna chyli was never found ruptured. This oedema in some animals became well established at the end of 24 hours, and persisted for one week, as was shown in a case in which a marked constriction, but not occlusion, of the thoracic duct was secured.

The lymph glands underwent marked hypertrophy. They had a fatty appearance, and showed the small follicles on the surface. They were very oedematous, and on section a considerable amount of clear, colorless fluid escaped. The increase in the size of lymph glands was more impressive in regions where they were ordinarily small and scarce, *e. g.*, between

the large mesenteric gland and the small intestine. Occasionally, glands of the size of almonds were seen in this region. In no case did the spleen seem larger than normal. The thoracic duct above the point of ligation was usually small and contracted.

In none of the dissections was the vagus nerve included in the ligature; occasionally, the left sympathetic chain was taken. Nor did the pigment reach the lungs via the direct lymphatic communication between the lungs and the cisterna chyli (Cunningham²¹).

The second type of collateral circulation is shown in Fig. 2, which was drawn from a specimen in which the subject was sacrificed 66 days after ligation. The thoracic duct with the entire lymphatic plexus was ligated. The azygos vein was included in the ligature and thus precluded any collateral vessel developing, as illustrated in Fig. 1. It is seen that an extensive lymphatic plexus has developed at the cisterna chyli and the azygos vein. Specimens of the contents of the azygos vein removed near the lymphatic-venous junction as well as a little further along the course of the vein, and examined under the microscope, revealed the Berlin blue pigment.

In one animal that was sacrificed three weeks after complete ligation, the lymphatic-venous connection was in the ninth left intercostal vein just before the latter entered the azygos vein. The communicating vessel took a tortuous path, commencing at a point on the thoracic duct at the level of the entrance of the ninth intercostal vein into the azygos vein, turned inferiorly and extended 7 mm. along the left side of the aorta, giving off small branches which entered the walls of that vessel; made several small sharp turns and extended about 4 mm. superiorly and laterally; then went medially and slightly inferiorly, making an acute angle with the ninth intercostal vein which it entered about 6 mm. from its junction with the azygos vein. This communicating vessel was almost the size of the intercostal vein, and was undoubtedly taking over the function of the thoracic duct.

In another case, a lymphatic-venous communication probably existed between a branch of one of the left lumbar veins and the cisterna chyli, since the blue pigment was found in the distal part of the lumbar vein, but in much greater quantity at the entrance of the lumbar vein into the inferior vena cava. As the connection was not actually seen, the absoluteness of the junction cannot be claimed.

RETROGRADE INJECTION OF LYMPHATICS

Before entering into the discussion of the above findings, attention is called to the retrograde injection of lymphatics as found in the majority of the dissections. In studying the lymphatic distribution in embryos by the injection method, advantage is constantly taken of the fact that specimens up to 5 cm. in length have the valves in their lymphatic vessels incompletely developed or entirely absent, and accordingly allow extensive retrograde injection of their lymphatic vessels (Sabin,²² Heuer,²³ Cunningham²⁴). In observing the relation of lymphatic vessels to connective tissue in pig fetuses by means of injections, MacCallum²⁵ noticed that the valves were

not very competent. In the adult, retrograde injections of lymph vessels entering the cisterna chyli and regional visceral lymph glands have been described by Schmidt-Mühlheim²⁶ and Bartels.²⁷ While injecting the mesenteric lymphatics, as above described, frequently one could see contributing branches of the vessel which was being injected centripetally gradually suffer retrograde injection through the successive dilatation of the intervalvular segments and the incompetence of their respective valves. In several cases such retrograde injections were carried to the subserous coat of the small intestine; while other cases showed a retrograde injection through the lymph glands draining the kidney with the afferent lymphatics injected as far as the hilum of that organ. Likewise, injections of small lymphatic vessels in the adventitia of the aorta and of vessels on the surface of the psoas muscle have been seen. Again, the large lymphatic plexus draining the liver and gall-bladder has been observed in a markedly dilated though uninjected state. In no case, however, have the parenchymatous lymphatic vessels been filled; and this in spite of the fact that these vessels are supposed to have either no or only a few valves (Bartels,²⁷ p. 71). The problem seems to lie in emptying lymphatic capillaries before injections are made; this condition may possibly be achieved by introducing hypertonic solutions into the blood stream, thus emptying the lymphatic capillaries and allowing the injection mass to enter. Waldeyer,²⁸ in 1867, called the attention of pathologists to the possibility of retrograde lymphatic transport of metastases.

DISCUSSION

A new method has thus been described for ligating the thoracic duct. While ligations of the duct have been performed in the neck and in the abdominal cavity by other workers, no attempts, it seems, have been made to secure ligation in the chest, although the duct has been wounded frequently in that cavity for experimental purposes (Boegehold²⁹). The operation can be performed without extensive dissection and without ligation of large veins (Schmidt-Mühlheim²⁶); it can be performed speedily and with a considerable guarantee of success. The method has provided experimental animals with absolute ligation that have lived apparently unaffected as long as 66 days after the operation. This period of survival of the animal in this study may be compared to the report of Lucibelli,³⁰ in which it was stated that the longest period of survival after ligation of the duct was three months and eleven days, but in this work of Lucibelli there is much reason to believe that the ligation was not absolute. However, from the above experimental results, one can conclude that the integrity of the thoracic duct is not essential to the life of the animal.

Furthermore, the method of injecting the mesenteric lymphatics avoids all possibility of injecting the venous system inadvertently, and has provided excellent examples of retrograde injections of the lymphatic vessels. Naturally, previous ligation of the thoracic duct may have played a considerable rôle in facilitating these injections.

It is interesting to note to what extent the present knowledge of the embryology of the lymphatic system may be util-

ized to explain the experimental findings. However, although it is not the purpose of this article to enter into the discussion of the various views regarding the origin of the lymphatic system (Huntington,¹⁰ Sabin,¹¹ McClure,¹² Lewis,¹³ Pensa¹⁴), it can be stated that at one time in embryonic life a large lymphatic plexus exists extending from the veins at the base of the neck on either side, down around the aorta, and terminating in the cisterna chyli; also, that the lower part of the embryonic thoracic duct is bilaterally symmetrical. The

branch leading from this plexus to the right thoracic duct may also be considered a part of the embryonic plexus; the fact that it enters a gland first before proceeding to the veins suggests that it was originally one of the smaller and more remote portions of the plexus. In another animal which was sacrificed 77 days after ligation, this small connecting vessel had a counterpart in two huge trunks, each of which was almost the size of a normal thoracic duct; the corresponding gland was also markedly enlarged. This response of embryological

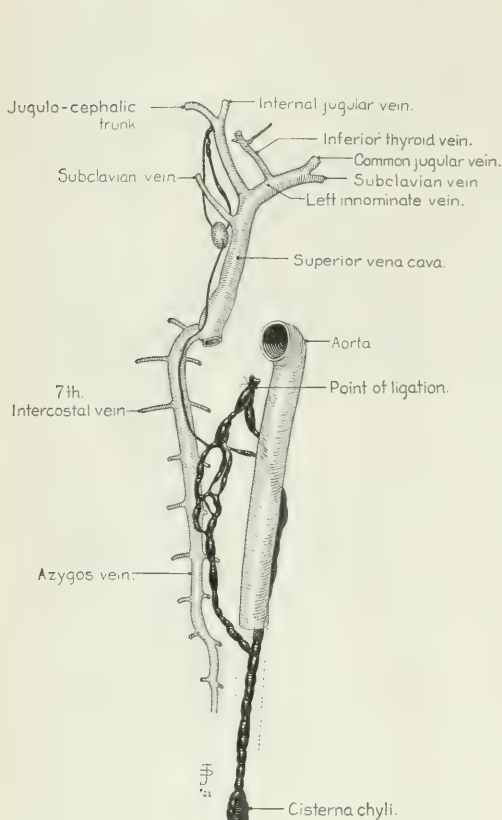


FIG. 1.

origin of the thoracic duct in the pig has been described by Baetjer.¹⁵ With these embryological data as a basis, Davis¹⁶ described and explained various types of thoracic duct anomalies, and it is interesting to note to what extent Fig. 1 fits in with some of the schematic representations of embryonic lymph channels that he depicted. Thus the small plexus developed below the point of ligation may well represent a portion of the embryological lymphatic plexus which normally does not function, but which, in response to the ligation, may have been forced to enlarge and dilate. Again, the small

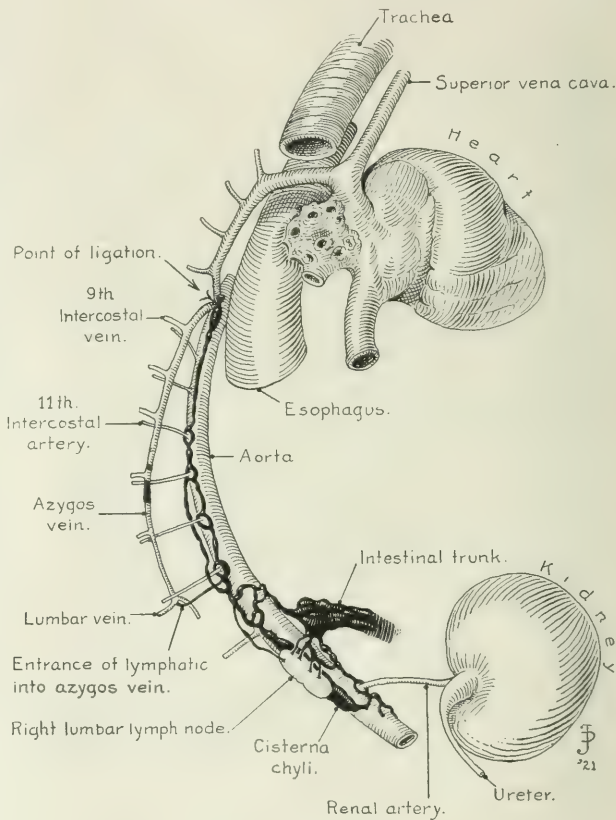


FIG. 2.

non-functioning lymphatic capillaries in a physiological capacity, if true, recalls in a measure the important work of Krogh¹⁷ in relation to blood capillaries.

On the other hand, it is impossible to explain with our present knowledge of embryology, the connection between the thoracic duct and azygos vein, as illustrated in Fig. 2. It seems as if the earliest stages in the development of lymphatic vessels are as yet unknown. The recent work of Sabin¹⁸ on the development of blood vessels would suggest that the differentiation of angioblasts as well as the sprouting of the vascular

endothelium could lead to the formation of lymphatics at other places along the venous system than from the anterior cardinal veins or from the veins of the Wolffian body. Thus, they could arise from the azygos vein, form a plexus with those lymphatic vessels arising from veins at the usual places, and gradually become small and functionless until physiological exigencies, produced, for example, by ligation of the thoracic duct, caused them to enlarge and function. Again, that large venous network in the early embryo which is associated with the azygos vein and which later disappears may be a factor in producing this lymphatico-venous connection.

E. R. Clark,¹¹ in the description of a very curious anomaly of the thoracic duct, considered the embryology of the lymphatic system in the explanation of the condition. Likewise, clinical cases of lymphatico-venous fistulae have been discussed on this basis (Halsted¹²).

Again, the establishment of collateral lymphatic circulation may occur through the regeneration of lymphatics. However, the studies in the regeneration of lymphatics have not been extensive enough, nor do they carry the necessary conviction (Meyer¹³). As much as one is inclined to believe in the regeneration of lymphatics—and this could easily explain the result as illustrated in Fig. 1—nevertheless, the necessary experimental proof seems lacking.

Since several cases of lymphatico-venous communications have been found (see Fig. 2), the subject of these connections is brought up for consideration. A host of writers (see Bartels¹⁴), as early as 1662, had described unusual lymphatico-venous connections; and numerous other investigators have attempted to disprove the evidence. Boddart,¹⁵ in 1899, described in the rabbit a connection of the thoracic duct with the azygos vein. Leaf¹⁶ in 1900 maintained that the azygos vein received many lymphatic vessels. However, Bartels,¹⁷ in 1909, questioned the correctness of any such lymphatico-venous communications. More recent work had thrown new light on the subject. Silvester¹⁸ described the presence of permanent communications between the lymphatic and the venous systems at the level of the renal veins in adult South American monkeys; Baum¹⁹ described cases where vasa efferentia of lymph glands emptied directly into veins; Job^{20,21} reported lymphatic communications with the inferior vena cava, ileolumbar, renal and portal veins in rats. It seems, then, well established that the lymph does not necessarily have to enter the venous system at the base of the neck.

The presence, then, of these lymphatico-venous connections enters into the problem of fat absorption. The entire anatomy of the chyliferous portion of the lymphatic system suggests that the newly absorbed fat avoids direct entrance into the liver. Biochemists, even with refined methods for determining fats in body fluids (Bloor²²), can recover from the thoracic duct only 60-70 per cent of the previously ingested food fats, but they are at a loss to explain the disposal of the remaining 30-40 per cent. Munk and Friedenthal²³ found that the blood fat increased after the thoracic duct had been tied. D'Errico²⁴ maintained that the percentage of fat in the portal vein was normally greater than that in the jugular vein, also that,

following ligation of the thoracic duct, the fat content of the portal vein with respect to the solid residue decreased but still remained higher than that of the jugular vein. He also believed that ligation of the duct accentuated lymphatico-venous communications. It is obvious that lymphatico-venous connections have been a generally unrecognized factor in all these studies of fat absorption involving ligation of the thoracic duct; and consequently these investigations are open to criticism.

Albrecht v. Haller, Albin and Hebenstreit are reported by Boegehold²⁵ to have seen the thoracic empty into the azygos vein. Wutzer,²⁶ while demonstrating the course of the thoracic duct in the body of a woman 37 years old, noticed that there were three connections between the duct and the azygos vein; also, that above these connections, the duct became attenuated and fibrotic. This case seems to show that in regard to this lymphatico-venous connection there is an analogous relationship between the human subject and the laboratory animal.

However, in spite of this analogy, one cannot claim with absolute surety that ligation of the thoracic duct in the human subject would prove equally innocuous. Yet it is clear, from the current surgical text-books as well as from the individual reports of injury to the thoracic duct, that there is no accepted method of treatment. Usually one of the four procedures is employed when the duct is injured: (1) Repair of the wounded duct and provision for subsequent ligation if necessary; (Cushing²⁷); (2) tamponade; (3) ligation of the vessel or (4) implantation of the severed duct into a vein (Harrison²⁸). Convalescence is the rule. Cushing considered that in case of ligation of the thoracic duct the lymph current would be reversed, and finally all the lymph would be taken up by the right thoracic duct—a postulate supported in a measure by these experiments. A record of numerous clinical cases involving injury to the thoracic duct with a discussion of their treatment has been given by Zesas.^{29,30} However, it is very questionable whether all the injuries to the thoracic duct really constituted injuries to the main trunk. Large lymphatic vessels from the head and neck often join the duct before its entrance into the vein and may be mistaken for the duct itself. Again, the presence of chyle does not guarantee the vessel to be the main trunk, because there frequently are several branches of the duct before its entrance into the veins.

In short, then, with the aid of the above experimental data, treatment of thoracic duct injuries would resolve itself into: 1, Repair of the injury if possible; or, 2, ligation. There is every reason to believe that similar treatment would apply to injury of other large and important lymph vessels; *e. g.*, the intestinal trunk.

SUMMARY

After reviewing the literature on the experimental ligation of the thoracic duct, an intra-thoracic method has been described for complete ligation of the thoracic duct in the cat. It seems that the integrity of the thoracic duct is not essential to the life of the animal. In some cases in which the ligation was absolute, collateral lymph circulation was established to the right thoracic duct; while in other cases which showed

complete ligation, lymphatico-venous connections were found to exist between the thoracic duct and the azygos vein. The embryology of the lymphatic system may explain partly, but not entirely, these findings which also have a bearing on studies of fat absorption as well as on the clinical aspect of injuries to the thoracic duct.

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STUDIES ON EXPERIMENTAL RICKETS

XV. THE EFFECT OF STARVATION ON THE HEALING OF RICKETS

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We have shown elsewhere¹ that certain types of faulty diets induce bone changes identical with those seen in rickets in human beings. Cod-liver oil exerts a pronounced effect in preventing the development of these changes, or, once they are established, tends to cause the bones to return toward a normal condition.

The most effective way which we have found to demonstrate the therapeutic value of cod-liver oil is through the application of our "line test," which has been fully described in another paper.² Young rats are restricted to our diet (Lot 3143) which has the following composition for a period of 35-40 days.

Lot 3143

Wheat	33.0
Maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
CaCO ₃	3.0

Any other diet having identical dietary properties would serve the same purpose.

This diet contains proteins of good quality and in great abundance (about 33 per cent), and about twice the calcium necessary for optimal growth and nutrition. It is, however, low in fat-soluble A and below the optimum in its content of phosphorus. One hundred grams contains 0.3019 grams of phosphorus.

If young rats are fed on this diet they develop a condition of the skeleton which is identical with the most severe rickets seen in the child. In the bones of these animals (Fig. 1) the cartilage is overgrown and abnormally persistent. The shaftward margin of the cartilage is irregular and the cartilage is invaded by blood vessels from the shaft of the bone. Lime salts are not deposited in the cartilage, so that no zone of provisional calcification is formed. A wide zone of tissue is formed between the cartilage and the shaft proper of the bone. This is made up of blood vessels, connective tissue and marrow elements, and of cartilage cells in all stages of degeneration, reversion, and of metaplasia into osteoid tissue. This is the rachitic metaphysis. The cortex of the shaft of the bone and the trabeculae of the spongiosa are surrounded by wide zones of osteoid tissue (uncalcified bone). No signs of abnormal resorption of bone tissue are to be found. These bones are very soft and at autopsy the animals show deformities of the skeleton which we have discussed in detail in a preceding communication.³

When a young rat has been restricted for 35-40 days to this diet, experience has shown that the cartilage and adjacent portion of the metaphysis in the long bones will be entirely free from calcium salts. If now cod-liver oil is administered to the amount of 2 per cent of the diet, calcium salts are deposited in the cartilage on the epiphyseal side of the metaphysis. This amounts to the reappearance of the provisional zone of calcification. From the work of Schmorl and others we know this phenomenon to be the beginning of the healing of the lesion. The deposited salts appear in longitudinal sections

of the bone as a fine line crossing the proliferative zone of the epiphyseal cartilage transversely.

We have found that the administration of cod-liver oil to the amount of 2 per cent of the diet causes the line of calcification to appear in the cartilage within five days.

We have recently observed that when young rats with rickets are made to fast for periods of three to five days (distilled water only being offered), healing begins in exactly the same way as it does when suitable amounts of cod-liver oil are administered.

Ten animals, as indicated in Table I, were fed on our diet No. 3143. At the expiration of the preparatory period they were placed in special cages and starved for 3-5 days.* They were then killed with chloroform and autopsied. The long bones were bisected longitudinally, immersed in a 1 per cent solution of silver nitrate, and examined under the microscope after exposure to light for the presence of a newly deposited provisional zone of calcification. The results of this gross examination were checked by the study of celloidin or frozen sections.

TABLE I

DATA DESCRIPTIVE OF RATS EMPLOYED FOR SHOWING THE EFFECTS OF FASTING ON THE INITIATION OF THE HEALING PROCESS IN RICKETS

Number of rats	Age in days when put on diet	Days on diet	Age in days at death	Days starved	Sex	Weight at death
1182	60	70	130	5	♂	82
1221	45	64	109	5*	♂	87
1341	45	49	94	2	♂	101
1345	55	49	104	3*	♀	85
1358	60	55	115	3	♂	125
1364	65	30	95	3	♂	80
1365	65	30	95	3*	♀	80
1366	65	30	95	3	♂	68
1368	60	35	95	3	♂	75
1369	60	35	95	3	♀	75

* Found dead on the morning of the day indicated.

The results of starvation were controlled by the examination of animals which had been raised in the same cages on diet No. 3143 at the same time, but had not been starved.† Not one of the starved animals failed to show reformation of the provisional zone of calcification, and other evidences of the healing of the rachitic process. None of the controls had a vestige of calcium in the cartilage or metaphysis.

Since we have found that even on diet No. 3143 rats which were exposed daily to sunlight in the summer from the begin-

* The cages employed for the fasting rats had wide mesh wire bottoms, so that the feces passed through, and it was impossible for the animals to eat their stools. No bedding of any kind was available for the rats to eat.

† We have sections from the bones of nearly 100 animals which were maintained on diet No. 3143 under the same conditions as our test animals. These served as an additional control for the test.

ning of the experiment did not develop rickets, three methods are now known of inducing the deposition of calcium salts in the cartilage of the bones of rachitic rats, viz., through starvation, exposure to light, and through the administration of cod-liver oil.

It is difficult to think of any single explanation for the common effect produced by cod-liver oil, sunlight and starvation on the skeleton of the rat rendered rachitic by means of a faulty diet. The possibility has been suggested that the effect of cod-liver oil may be attributable to physical force of one kind or another. This view we have discussed more fully in another place. But even if the effect of cod-liver oil should be the result of a physical force rather than a chemical reaction it does not follow that one could generalize regarding the other vitamins and accept the view that they necessarily act in an analogous manner. The analogy between the effects on the body of vitamin preparations and of the hormones is so striking that we are not justified in hastily abandoning the view that the vitamins are organic substances. Two at least of the hormones, thyroxin and adrenalin, are definite organic compounds.

Starvation causes healing of the rickets in a rat just as do cod-liver oil and sunlight, and the mechanism of the deposition of calcium salts in the proliferative cartilage may be the same. But cod-liver oil and sunlight improve the general condition of the animal. This is indicated by increased growth, longevity, muscular activity, storage of fat, and sexual potency. Starvation, on the other hand, causes the death of the rat in a few days. Its effects on the animal are favorable only in that they cause the healing of rickets. The mode of action of the three agents must be entirely different. It seems necessary to think that sunlight and cod-liver oil act by enabling the cells to carry on, even when their food supply is unfavorable. In other words, they aid the organism to adapt itself to an adverse environment. Starvation, on the other hand, must produce healing of the rickets at the expense of other body tissues. We cannot disprove at present the possibility that the cell functions more effectively when it is relieved of the load which a faulty diet imposes on it. However, during starvation certain tissues are destroyed for the upkeep of the supply of nutriment for those whose integrity must be safe-guarded to preserve life.

We have shown that the administration of cod-liver oil is followed by healing of rickets in rats even when they are receiving a diet with a faulty calcium-phosphate ratio. It may be that during the disintegration of protoplasm, which is consequent upon starvation, some substance is liberated which permits the animal to readjust the salt ratio within itself. But although it is possible that starvation liberates from the tissues an organic factor or factors, such as are in cod-liver oil, it is not necessary to account for healing in this way. Providing calcium and phosphorus are present in the proper proportions, normal calcification of the skeleton will occur. When the defects in the diet are such as they happen to be in the diet which we employed in these experiments (diet No. 3143), it would only be necessary for phosphorus to be liberated into the blood stream from disintegrated protoplasm (muscle for ex-

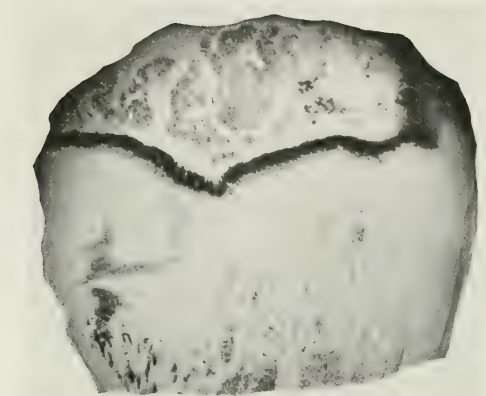


FIG. 1.—Photomicrograph of a section from the rachitic animal which had received diet No. 3143. There is no calcification of the epiphyseal cartilage or of the metaphysis.

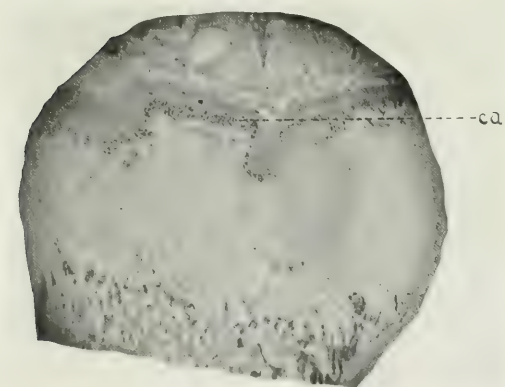


FIG. 2.—Photomicrograph showing the re-formation of a provisional zone of calcification (Ca) in the bone of a rachitic animal which had been starved for 5 days.

ample) in order to cause calcium deposition to occur in the cartilage. Animals on diet No. 3143, like rachitic children, have much less than the normal amount of phosphorus in the circulating blood. The administration of cod-liver oil or radiation from the mercury vapor quartz lamp causes the phosphorus in the blood to rise and it is not improbable that the healing of rickets which results from starvation is the immediate result of an equivalent increase of this element in the circulating blood. Just as soon as the load of a defective diet is removed and the body is forced to draw on its own tissues for maintenance of life and function, stored foodstuffs are released into the blood stream as the result of a process of selective tissue decomposition.

These experiments furnish the first anatomical proof of the beneficial effect of starvation on the animal body. The good effects of fasting are given a new meaning, because the organism is able to adapt itself to pathogenic distortions of normal metabolic ratios when the burden of carrying on exogenous metabolism is removed. Since the starving body is capable of readjusting abnormal relations within itself it is easy to understand the benefit derived by a diabetic from occasional hunger days, and why it is that the wasted athreptic infant does not develop rickets.

Rickets has certain of the characteristics of a deficiency disease because a certain substance contained in cod-liver oil and elsewhere corrects an anatomical condition which develops when the calcium and phosphorus in the diet are present in wrong proportions. Yet rickets has a feature entirely distinct from beri-beri, scurvy, and xerophthalmia. The relation between two inorganic elements determines the extent of the animals' need for the organic factor which cod-liver oil furnishes. No such relationship between a vitamin and any other food substance has been clearly demonstrated in any other condition.

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NOTES AND NEWS

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IMMUNOLOGICAL REACTIONS OF BENCE-JONES PROTEINS

I. DIFFERENCES BETWEEN BENCE-JONES PROTEINS AND HUMAN SERUM PROTEINS

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In a previous paper,¹ presenting a preliminary account of our study of the immunological reactions of Bence-Jones proteins, we have reported that by the use of the precipitin, complement-fixation and anaphylactic reactions we have been able to show that differences exist between the so-called Bence-Jones protein and the proteins of human serum, and also between preparations of Bence-Jones protein from various sources. While the specimens of Bence-Jones protein which we studied were all typical as regards their phases of coagulation and solution by heat in an acid medium, they differed considerably in their physical properties and in their immunological reactions. These differences were indeed so great that we were forced to regard the so-called "Bence-Jones body" as a group of similar, but not identical, substances, and to refer to the group as the Bence-Jones proteins. One of these proteins (No. 4) crystallized spontaneously in the urine of the patient who excreted it. This property permitted purification of the substance by recrystallization. An account of this patient (R) with a description of the protein has been published by Walters² from the Mayo Clinic, where the patient was under observation on the service of Dr. Rowntree. The other specimens of Bence-Jones proteins used by us were

isolated from urine by various methods of precipitation. They will be described in detail in our paper dealing with their specific immunological differences. In the present report, we shall give the evidence in support of the contention that it is possible by the use of immunological methods to differentiate Bence-Jones proteins from the proteins of human serum.

With the more frequent use of purified proteins in immunological studies, an increasing number of them are being found to possess individual antigenic specificity. It has been known for a long time that the proteins of the lens of the eye and of spermatozoa have this characteristic, and more recently Wells and Osborne,³ using purified vegetable proteins, Woods⁴ using the pigment from the uveal tract, and Dale and Dakin⁵ with crystalline albumin from the eggs of hen and duck, have established the fact that chemical composition, rather than biological origin, is the basis of the specificity of these substances. The chemical and physical peculiarities of Bence-Jones proteins and their absence from the blood and urine of normal persons have conveyed the obvious suggestion that it might be possible by immunological studies to draw a distinction between the blood-proteins and Bence-Jones proteins. This phase of the question has occupied the attention of a number of investi-

gators. The results, however, of the experiments of all except two students of this problem have been reported as indicative of an identity rather than a difference between these proteins.^{9, 7, 8, 9} From accounts of the way in which the experiments were conducted, and from the results of our work, it seems that the uncritical use of mixtures of proteins has led to these equivocal results. The most notorious example of this is the work of Abderhalden and Rostoksi,⁶ which has influenced many of the subsequent immunological studies of Bence-Jones proteins and the opinions upon the nature of Bence-Jones proteinuria. The patient studied by Abderhalden had chronic nephritis as well as multiple myelomata. As a consequence of the two diseases he excreted in his urine Bence-Jones protein and proteins which Cameron and Wells,²⁰ in studies of the urine in nephritis, have shown to be identical with the proteins of human serum. Abderhalden injected the whole urine of this patient into a rabbit, and obtained an immune serum which precipitated both Bence-Jones protein and human serum. In drawing his conclusions, however, he neglected the possibility that he was dealing with a mixture, and developed his ideas about the identity of Bence-Jones protein and serum proteins as if he had been using single pure antigens. It now seems probable that unless a Bence-Jones protein can be separated from urine by crystallization, and purified by recrystallization, it is impossible to separate it by any method of fractional precipitation from other proteins which are usually present with it in the urine.

The investigators who have succeeded in finding immunological differences between Bence-Jones proteins and serum proteins are Massini²¹ and Hektoen.²² By means of the complement-fixation test, Massini was able to show that an anti-serum to the preparation used by him fixed complement in the presence of higher dilutions of the Bence-Jones protein than of human serum. Hektoen's recently published preliminary note reports that by absorption of precipitins specific reactions can be obtained which sharply differentiate Bence-Jones proteins from the proteins of human serum, even when mixed antigens are used.

REPORTS OF EXPERIMENTS

In our experiments to discover, if possible, immunological differences between Bence-Jones proteins and the proteins of human serum, we used precipitin and complement-fixation reactions with the sera of rabbits immunized to human serum and to several preparations of Bence-Jones proteins, and anaphylactic reactions of guinea-pigs sensitized to these substances.

PRECIPITIN REACTIONS

The precipitin-sera were prepared as follows:

Anti-human serum. Rabbit No. 161 immunized to human serum by 7 intravenous injections at intervals of 2 to 6 days. The first dose was 0.5 c. c., the last 12 c. c. of serum. Ten days after the last injection, the animal was bled, when its serum gave a precipitate with a 1-1000 dilution of human serum.

Anti-Bence-Jones protein sera: Rabbit No. 144 was immunized to the crystalline Bence-Jones protein (No. 4) by six intravenous injections

of a 1 per cent solution of No. 4 at intervals of 4 to 6 days. The first dose was 2 c. c., the last 20 c. c. Ten days after the last injection, when the rabbit's serum caused a precipitate in a 1-1,000,000 dilution of a 4 per cent solution of No. 4, the animal was bled.

Rabbit No. 153 was injected intravenously at intervals of 4 to 6 days with amounts of a 0.5 per cent solution of Dr. J. Rosenbloom's preparation of Bence-Jones protein increasing from 3 c. c. to 20 c. c. Six days after the fifth injection, the rabbit was bled. Precipitin titer 1-100,000 (antigen dilution).

Rabbit No. 189 was immunized to a preparation of Bence-Jones protein designated as "Simpson," given us by Dr. C. G. Guthrie. After 8 injections of amounts of 2-6 c. c. of a 2 per cent solution of this protein, at intervals of 3 to 5 days, the precipitin titer of this animal was 1-2000 (antigen dilution).

Rabbit No. 195 was immunized to a preparation of Bence-Jones protein denoted as "Farrel," and given to us by Dr. C. G. Guthrie. At intervals of six days, 1 c. c., 5 c. c., and 7 c. c. of a 3 per cent solution of this protein were injected intravenously. Five days after the last injection, when the animal was bled, the titer of the serum was 1-8000 (antigen dilution).

With the exception of No. 4, which was the crystalline Bence-Jones protein, the other Bence-Jones proteins were non-

TABLE I
PRECIPITIN REACTIONS

Antihuman serum 161. Precipitation with antigen	Dilution of antigen			
	0	1-10	1-100	1-1000
Human serum.....	++++	+++	++	+
Bence-Jones protein (4 per cent solution).
No. 4 (crystalline)	0	0	0	0
No. 1.....	+	0	0	0
Coag. No. 2.....	0	0	0	0
Coag. R. urine.....	+	0	0	0
R. urine.....	++	+	0	0
Taylor	++	+	0	0
Farrel	++	+	+	0
Simpson (2 per cent).....	0	+	++	0
Dine	+	+	0	0
Dine urine	+	++	+	0
X.....	++	+	0	0
X urine	+++	++	+	0
Rosenbloom	+	+	0	0

crystalline, and had been isolated from urine by various methods of precipitation.

The precipitin tests were done in clean sterile tubes, with clear solutions of the sera and antigens. False results due to bacterial growth were eliminated. The antigens, in various dilutions, were layered upon the anti-sera, and the first reading was made by noting the presence or absence of precipitate at the plane of junction of the two fluids one hour after their apposition. The fluids were then mixed, placed in the incu-

bator at 37° C. for 24 hours, and a second reading taken of the sediment in the bottom of the tubes. The controls, which are omitted from the following tables, were made by mixing equal amounts of each component with normal salt solution and incubating these mixtures with the series of tests. When precipitate occurred in the controls, the corresponding tests were discarded or repeated with fresh solutions. In the tables, the signs indicate the amount of precipitate formed; 0 denoting no precipitate, + + + + a very heavy precipitate. The results of the precipitin reactions with antihuman serum are summarized in Table I.

The advantage gained from the use of a pure crystalline protein is at once apparent from the data presented in Table I. The antiserum to human serum proteins does not precipitate the crystalline preparation of Bence-Jones protein. On the other hand, antihuman serum forms a precipitate with all non-crystalline Bence-Jones proteins which have been salted-out

TABLE II
PRECIPITIN REACTIONS

Serum	Precipitation with	Dilution of antigen					
		0	1-10	1-100	1-1000	1-2000	1-100,000
144. Anti-Bence-Jones, No. 4.	Bence-Jones, No. 4 (4 per cent.)	0	+	+++	+++	++++	+++
	Human serum...	0	0	0	0	0	0
153. Anti-Bence-Jones, Rosenbloom.	Bence-Jones, Rosenbloom (4 per cent.)	0	+	++	+++	+++	+
	Human serum...	0	+	++	+++	+	0
189. Anti-Bence-Jones, Simpson.	Bence-Jones, Simpson (2 per cent.)	+++	+	++++	+++	+	0
	Human serum...	++	+	0	0	0	0
195. Anti-Bence-Jones, Farrel.	Bence-Jones, Farrel (4 per cent.)	+++	+	++++	+++	++	0
	Human serum...	+	++	+	0	0	0

or otherwise precipitated from urine. With the whole urine from patients with multiple myelomata, containing presumably more protein identical with the proteins of serum, the precipitation by antihuman serum is greater than with the fractions of these urines containing the Bence-Jones protein.

In Table II are summaries of precipitin reactions with human serum, antisera to several preparations of Bence-Jones proteins and their homologous antigens.

From Table II it is seen that a very potent antiserum to the crystalline Bence-Jones protein does not precipitate human serum, while the antisera to the other less pure preparations of Bence-Jones proteins give "cross-reactions," precipitating both their homologous antigens and human serum.

As Hektoen¹³ has pointed out, the method of absorption of precipitins can be used, when mixtures of proteins are unavoidable, to demonstrate differences between serum proteins and Bence-Jones proteins. There is, however, much to be learned about this method before the results obtained with it can be correctly interpreted.^{13, 14} In its use, we encountered a number of difficulties. In the first place, precipitating sera, such as those used by us, are no longer active when diluted more than 1 to 20, or 1 to 40. This places a narrow limit upon the

applicability of the method, as it may be necessary during the process of absorption to dilute the serum beyond its effective precipitin-concentration. When dilution of the serum is avoided, the so-called "prozone" may interfere with the formation of a precipitate during the phase of absorption. In many mixtures of undiluted serum and a concentrated solution of its protein antigen no precipitate occurs, yet when the mixture is diluted, the precipitate appears. In this case, of course, neither the antigen nor antibody is removable by centrifugation, and the delayed precipitation due to the first reaction often occurs when salt solution or the diluted antigens to be tested are added subsequently. It may be that the hydrogen-ion concentration of the fluids is a factor of prime importance in the precipitin reaction. A concentration of hydrogen-ions in the region of the isoelectric point of serum globulin may permit a precipitin reaction to progress to completion even in mixtures of undiluted immune serum and concentrated solutions of proteins. We have not investigated this point thoroughly. The results, however, of our experiments with the absorption of precipitins are unequivocal when the "ring-test" is used, readings of the precipitate at the junction of serum and antigen solutions being taken within one or two hours after the one has been layered upon the other. When allowed to stand for 18 hours or more, a slow precipitation

TABLE III
ABSORPTION OF PRECIPITINS

Anti-serum	Absorbed with	Precipitation with	Dilution of antigen					
			0	1-10	1-100	1-500	1-1000	1-2000
Anti-human serum 161.	Bence-Jones, No. 4.	Human serum.	++++	++	++	+	0	..
		Bence-Jones, No. 4.	0	0	0	0	0	..
		Simpson	+	+	+	0
	Bence-Jones, Simpson.	Farrel	+	+	+	0
		Human serum.	+++	++	++	+	0	..
		Bence-Jones, No. 4.	0	0	0	0	0	..
	Bence-Jones, Farrel.	Simpson	0	0	0	0
		Farrel	0	+	±	0
		Human serum.	++	++	+	±	0	..
	0.85 per cent salt solution.	Bence-Jones, No. 4.	0	0	0	0
		Simpson	+	+	+	0
		Farrel	+	+	+	0
Anti-Bence-Jones, Farrel, 195.	Human serum	Bence-Jones, Farrel.	+++	++++	+++	++	++	+
		Human serum.	0	0	0	0
	0.85 per cent salt solution.	Bence-Jones, Farrel.	+++	++++	+++	+++	+++	++
		Human serum.	0	+	±	0

often occurs in all sera, including the controls, showing that the original reaction had not been complete but was continuing under the conditions permitted by the subsequent dilution.

The technic used in absorbing the precipitins was as follows: With precautions to prevent bacterial growth in the tubes, 1 c. c. of antiserum was mixed with 1 c. c. of a 2 or 4 per cent solution of a Bence-Jones protein. This mixture was incubated at 37° C. for 24 hours, placed in the ice-chest for 18 hours and then centrifuged until clear. The usual series of tests and controls were then made with this absorbed serum, diluted one to two as a consequence of the addition to it of an equal quantity of the antigen for the phase of absorption. A control to determine the effect of simple dilution and manipulation upon the antiserum was made by carrying a mixture of equal parts of immune serum and salt solution through the stages of incubation and absorption.

Antihuman serum No. 161 was absorbed in this manner with 4 per cent solutions of the crystalline Bence-Jones protein, and the Farrel preparation of Bence-Jones protein, and with a 2 per cent solution of the Simpson Bence-Jones protein. To show the effect of the reversal of this procedure, an antiserum to the Farrel specimen of Bence-Jones protein was absorbed with human serum. The results of these absorption tests are summarized in Table III.

Several of the experiments summarized in Table III show again that antihuman serum does not precipitate the crystalline preparation of Bence-Jones protein (No. 4) and, further, that this Bence-Jones protein does not absorb precipitin from antihuman serum. The data demonstrate that by absorption of antihuman serum with certain preparations of Bence-Jones proteins it is possible to remove from this immune serum the precipitin responsible for the flocculation with solutions of these preparations, while only slightly lowering the titer of the serum for its homologous antigen, human serum. Confirmation of the result is obtained when an antiserum to one of these preparations of Bence-Jones protein (Farrel) is absorbed with human serum. The conclusion seems inevitable that some of these non-crystalline preparations of Bence-Jones proteins contain traces of human serum proteins, while, in this respect, the crystalline Bence-Jones protein acts as a single antigen.

COMPLEMENT-FIXATION REACTIONS

For the complement-fixation reactions antihuman serum was tested against the various Bence-Jones proteins, and antisera to the crystalline and Rosenbloom Bence-Jones proteins were tested against human serum. Preliminary titrations were made with all sera and solutions of the proteins to determine their anticomplementary action, and in the tests the anticomplementary controls contained twice the amount which did not inhibit hemolysis. This required the use of sera diluted one to two, and 1 to 20 dilutions of the solutions of Bence-Jones proteins. These dilutions, as has been pointed out, are just on the limit of effective concentration of precipitin. The results of the complement-fixation tests, therefore, indicate broad relationships, as closer interactions are lost through the dilution. In the tests, 0.25 c. c. was the unit volume of each component used. The usual antishoop amboceptor, with a titer of 1 to 3200, and guinea-pig serum as complement were employed. After the mixtures of antiserum, protein solution

and complement were made, they were incubated in the water-bath at 37° C. for one hour. At the end of that time, three units of amboceptor were added, the 2.5 per cent suspension of sheep cells placed in the tubes, and all returned to the water-bath. Readings were taken at the end of one hour, or when all the proper controls had cleared. The results of these tests are collected in Table IV, in which + + + + indicates complete fixation (absence of hemolysis), and lesser degrees of fixation by proportionally fewer + signs.

The complement fixation reaction, according to this method, combines a differentiation based upon dilution of the antisera and antigens with the differences due to fixative power of

TABLE IV
COMPLEMENT FIXATION REACTIONS

Serum	Antigen	Conc.	Dilut.	Result
Anti-human serum, 161. Diluted 1-2.	Human serum.....	..	1-40	++++
	Bence-Jones protein:			
	No. 4.....	4%	1-20	0
	No. 1.....	4%	1-20	0
	Coag. R. urine.....	4%	1-20	0
	R. urine.....	..	1-20	+
	Taylor.....	4%	1-20	+
	Farrel.....	4%	1-20	+
	Simpson.....	2%	1-20	0
	Dine.....	4%	1-20	0
	X.....	4%	1-20	0
Anti-Bence-Jones, No. 4. Serum 144. Diluted 1-2.	Rosenbloom.....	4%	1-20	+++
	Bence-Jones, No. 4...	4%	1-20	++++
Anti-Bence-Jones, Rosenbloom. Serum 153. Diluted 1-2.	Human serum.....	..	1-20	0
	Bence-Jones, Rosen- bloom.	4%	1-20	++++
	Human serum.....	..	1-20	0

specific precipitates. Its effect, therefore, is to indicate only broad relationships. The results thus obtained, as presented in Table IV, confirm the results of the precipitin reactions in showing immunological distinctions between Bence-Jones proteins and the proteins of human serum.

ANAPHYLACTIC REACTIONS

Anaphylactic reactions were studied upon the guinea-pig as a whole and on excised uterine horns of young virgin guinea-pigs. The animals in this series were actively sensitized, except where otherwise stated, by an intravenous injection of 0.25 c. c. of human serum or of a 4 per cent solution of a Bence-Jones protein. After an interval of approximately three weeks, the animals were tested by an intrajugular injection of serum or Bence-Jones protein. The data of these experiments are presented in Table V.

Anaphylactic reactions with the crystalline Bence-Jones protein were not as sharp as we desired, as it was difficult actively to sensitize guinea-pigs to this preparation. Both large and small doses of the crystalline protein were given on the first intravenous injection, and the animals were tested by a second intravenous injection of larger amounts after intervals of 18 to 27 days. With one series, fairly satisfactory results were obtained. The guinea-pigs of this lot received as the sensitizing dose injected intravenously 0.25 c. c. of a 4 per cent solution of the crystalline Bence-Jones protein (No. 4) on January 27, 1921. On February 14, 21 days later, an intravenous injection of 1 c. c. of the 4 per cent solution of No. 4 produced a severe but not fatal shock, in guinea-pig No. 396. None of 10 guinea-pigs thus sensitized to Bence-Jones protein No. 4 showed any reaction to human serum.

TABLE V
ANAPHYLACTIC REACTIONS

Guinea pig	Sensitizing dose	Interval	Intoxicating dose	Result
250	Human serum 0.25 c. c.	18 days	Human serum 0.25 c. c.	Typical anaphylaxis. Death, one min.
151	Human serum 0.25 c. c.	18 days	Bence - Jones No. 4. 2 c. c. 0.5% sol. 10 minutes later: 2 c. c. human serum intraperit.	No reaction. Typical shock. Death.
253	Human serum 0.25 c. c.	18 days	Bence - Jones No. 1. 7 c. c. 1% sol. 10 minutes later: 1 c. c. human serum intraven.	No reaction. Typical shock. Death.
251	Human serum 0.25 c. c.	18 days	Bence - Jones. Rosenbloom prep. 1 c. c. 4% sol.	Slight, definite reaction. Survived.
252	Human serum 0.25 c. c.	18 days	Bence-Jones, Taylor's prep. 1 c. c. 4% sol.	Slight, definite reaction. Survived.
173	Bence - Jones No. 1. 2 c. c. 0.5% sol.	27 days	Human serum 0.25 c. c.	Typical shock. Death, one min.
275	Bence - Jones. Rosenbloom prep. 1.5 c. c. 1% sol.	18 days	Human serum 0.25 c. c.	Typical shock. Death, one min.
290	Bence-Jones, Taylor's prep. 1 c. c. 1.5% sol.	18 days	Human serum 0.25 c. c.	Typical shock. Death, two min.

The anaphylactic reactions summarized in Table V and in the above paragraph demonstrate a complete difference between the crystalline Bence-Jones protein and the proteins of human serum. On the other hand, guinea-pigs sensitized to human serum were also sensitive, though in less degree, to the Rosenbloom and Taylor preparations of Bence-Jones proteins, and guinea-pigs sensitized to these Bence-Jones proteins and to Bence-Jones protein No. 1 were extremely hypersensitive to human serum. From the results of the precipitin reactions, we are led to assume, in explanation of the crossed anaphylactic shocks, that the non-crystalline preparations of Bence-Jones proteins (No. 1, Taylor and Rosenbloom) contain traces of human serum proteins. These traces of serum proteins are sufficient to sensitize the animal to human serum, so that when a large amount of human serum is administered in the second injection, fatal anaphylactic shock occurs. Their content of serum proteins, however, is too small to produce a fatal reaction when they are injected in the amounts used by us into guinea-pigs primarily sensitized to human serum.

An analysis of these preparations of Bence-Jones proteins was permitted by the graphs obtained by the Schultz-Dale method.¹⁵ The apparatus and solutions used in our studies of the anaphylactic reactions of isolated uterine muscle were essentially those described by Dale. Virgin guinea-pigs, sensitized to human serum or Bence-Jones proteins, were killed by a blow on the head about three weeks after the first injection. The uterus was removed, one horn was attached to the lever of a kymograph, and this strip of smooth muscle was then submerged in Ringer-Locke solution. The bath, 50 c. c. of Ringer-Locke solution, was kept at 37-38° C., and was supplied con-

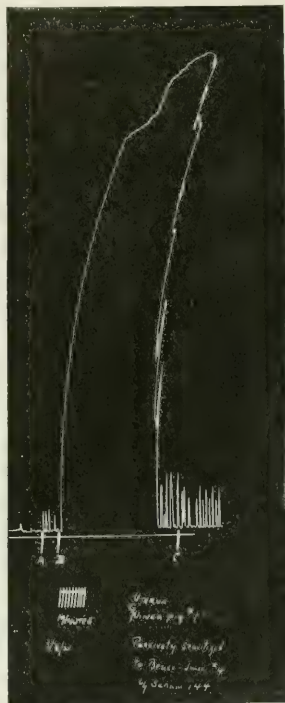


FIG. 1.—Uterus of guinea-pig No. 1. Passively sensitized to Bence-Jones protein No. 4 by injection of 1 c. c. serum 144 into peritoneal cavity. Killed 18 hours later.

At A: 1 c. c. human serum.

At B: 1 c. c. 3% sol. Bence-Jones protein No. 4.

At C: 1 c. c. 3% sol. Bence-Jones protein No. 4.

tinuously with oxygen. Tracings of the movements of the strip of uterus were recorded on a smoked drum.

It was found more satisfactory passively to sensitize guinea-pigs to the crystalline Bence-Jones protein. This was easily accomplished by injecting intraperitoneally 1 c. c. of the serum of rabbit No. 144, which had been immunized to this Bence-Jones protein. The precipitin titer of this serum was 1 to 1,000,000, in terms of dilution of the antigen. Eighteen hours later, these guinea-pigs were found by tests to be hypersensitive to Bence-Jones protein No. 4.

Representative graphs, Figs. 1, 2 and 3, illustrate the results of some of the anaphylactic reactions with the isolated smooth muscle of the uterine horns of sensitized guinea-pigs. From Fig. 1 it is seen that the uterus of a guinea-pig sensitized to the crystalline Bence-Jones protein No. 4 did not react to human serum, and Fig. 2 shows that the uterus of a guinea-pig sensitized to human serum was not affected by the solution of this crystalline protein. Fig. 3 is of especial interest, as it is analogous to experiments on the absorption of antibodies. The uterus used in this test was from a guinea-pig actively sensitized to Rosenbloom's non-crystalline preparation of Bence-

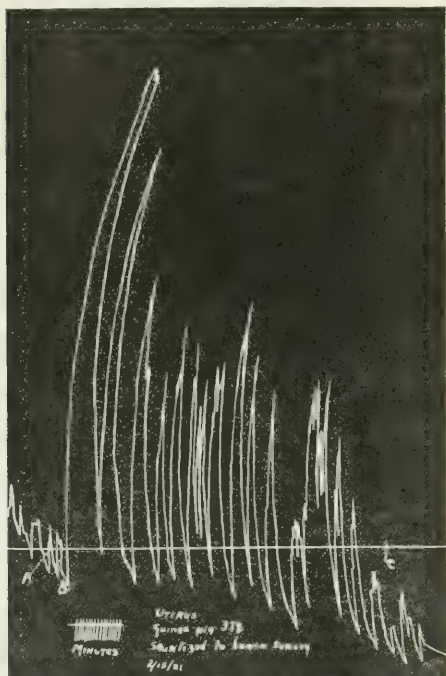


FIG. 2.—Uterus of guinea-pig No. 378, sensitized to human serum.

At A: 1 c. c. 4% sol. Bence-Jones protein No. 4.
At B: 1 c. c. human serum.
At C: 1 c. c. human serum.

Jones protein. When treated with a solution of this Bence-Jones protein, a contraction occurred. A subsequent application of the same protein failed to cause a contraction, showing that the muscle was desensitized to that antigen. It, however, remained hypersensitive to human serum, as shown by the next phase of the curve, which records the contraction produced by the addition of human serum to the bath. After this second contraction, the uterus was specifically desensitized also to human serum. This analysis permits the definite conclusion that the Rosenbloom preparation of Bence-Jones protein is a mixture of a Bence-Jones protein and human serum proteins.

SUMMARY AND CONCLUSIONS

In the possession of a crystalline Bence-Jones protein we had at our disposal an ideal substance for immunological studies. By crystallization, it could be freed from possible traces of serum proteins and thus permitted the use of a purified preparation to obviate the confused results which vitiate many immunological experiments. Its quality as an antigen was easily established, and the reactions dependent upon its antibodies were unequivocal. In contrast to this, the non-crystal-

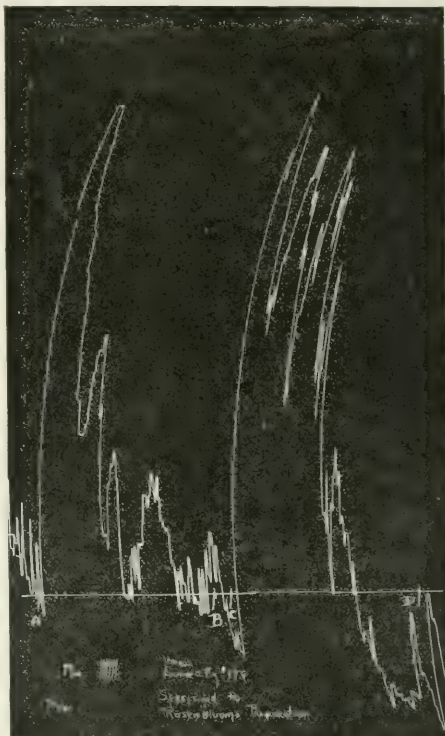


FIG. 3.—Uterus of guinea-pig sensitized to Bence-Jones protein (Rosenbloom).

At A: 1 c. c. 4% sol. Bence-Jones protein (Rosenbloom).
At B: 1 c. c. same solution.
At C: 0.5 c. c. human serum.
At D: 0.5 c. c. human serum.

line preparations of Bence-Jones proteins, precipitated from the urine by fractionation with salts or heat, gave the "cross" reactions usually obtained with mixed antigens. Comparisons between the Bence-Jones proteins and the proteins of normal human serum were made by the use of precipitin, complement-fixation and anaphylactic reactions. The precipitin reactions were extended by the method of the absorption of antibodies and the anaphylactic reactions were submitted to analysis by the Schultz-Dale method of the graphic record of the contrac-

tion of smooth muscle. The results of all these experiments were in accord, and allow the following conclusions to be drawn:

1. The crystalline Bence-Jones protein acts as a single antigen.
2. The non-crystalline preparations of Bence-Jones proteins, isolated from the urine by salting-out or other precipitation methods, contain traces of serum proteins.
3. The Bence-Jones proteins are immunologically different from the proteins of normal human serum.
4. These differences between proteins from the same animal are further evidence in support of the conception that the specificity of proteins is not dependent upon their biological origin, but due to their chemical constitution.

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YAWS

AN ANALYSIS OF 1046 CASES IN THE DOMINICAN REPUBLIC

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HISTORICAL SKETCH OF SANTO DOMINGO

The island occupied by the Republics of Santo Domingo and Haiti is the second largest of the West Indian group lying between Cuba and Jamaica on the west and Porto Rico on the east. It lies between the 17th and 20th parallels of north latitude and between the 68th and 74th meridians.

Its 28,249 square miles of area are diversified by almost every variety of topography; fair and fertile valleys, broad savannahs, towering mountains, sinister desert. The northern half or two-thirds of the island is traversed by two almost unbroken mountain ranges, the Monte Christi and the Cibao or Cordillera, between which stretches from the sea on the west to Samana Bay on the east a beautiful valley, the "Vega Real," the eastern half of which is well watered and wonderfully fertile. It is in this valley and on the lower slopes of the adjacent mountains that most of the cacao and tobacco grow, which, with sugar, make up the chief exports of the island. Indeed the cacao crop of Santo Domingo is said to be the third largest of any country of the world. The southeastern part of the island is occupied by a broad coastal plain extending back from the Caribbean as much as 50 miles. This is occupied by broad savannahs, well watered and covered with a luxuriant growth of grass which would furnish excellent pasturage for vast herds of cattle. It is here that the great sugar estates are located which produce the largest single

article of commerce of the island. Coffee is grown commercially to some extent in the mountains of the southwestern part of Santo Domingo toward the Haitian border.

Tropical fruits—bananas, plantains, pine-apples, coconuts, oranges, lemons, limes, mangoes, aguacates, bread-fruit—flourish. Birds of brilliant plumage rival the gorgeous tropical flora in beauty. The fauna is curiously limited in some respects, the largest native mammal on the island being the agouti, a shy little beast somewhat resembling the guinea-pig in appearance and about the size of a rabbit.

The heat of the tropics is tempered along the coast by sea breezes, and by the altitude of the mountains in the interior, the highest peak, Loma Tina, rising 10,200 feet above sea level.

The commercial life of the island centers in the various ports scattered along the coast—Monte Christi, Puerto Plata, Samana, Sanchez, La Romana, Maccoris, Santo Domingo City and Barahona. The number of inhabitants in these towns varies from a few hundred in the smaller up to about 25,000 in Santo Domingo City, the capital.

The population of Santo Domingo, which occupies the eastern three-fourths of the island, is estimated at between 700,000 and 800,000 inhabitants, while Haiti, occupying the western fourth of the island, has a population of about two and a half million. Haiti is known as the Black Republic,

and takes pride in this appellation as indicating pure blood. The language is French. Santo Domingo, on the other hand, does not boast of its negro blood, although it is probable that considerably more than 75 per cent of the population possess it. In the larger towns, especially on the coast, there is a small white population, which shades down through all gradations of color to pure black. Few whites live in the interior, except in the occasional inland towns, the largest of which is Santiago de los Caballeros, a flourishing city of about 20,000 inhabitants, beautifully situated in the Vega Real. The language of Santo Domingo is Spanish.

Life in the interior is simple and furnishes little incentive for industry. The requirements for shelter and clothing are simply met and nature furnishes an adequate and healthful diet, with little exaction beyond the effort required to take it.

Perhaps few of us realize or remember the important part played by this obscure island in the history of Western civilization. Discovered on December 6, 1492, by Christopher Columbus, its present capital, Santo Domingo City, is the oldest existing European settlement in the Western Hemisphere. At the time Columbus landed and took possession of the island in the name of the King and Queen of Spain, calling it La Española, the native population consisted of Arawak Indians, estimated at between two and three million souls. Columbus described them as a mild and peaceful race, characterized by their sweetness of temper, the men of hideous visage but the women comely. They offered little resistance to the Spanish occupation, and their land was given by the King of Spain in huge grants to his favorites, who, attracted by the glowing descriptions of the wealth and beauty of the country, came over in large numbers, built palaces and cathedrals, set up a vice-regal court and lived in an extravagance which almost rivaled that of the court of Spain.

The Indians were easily subjugated, quickly reduced to a state of slavery, and under Spanish tyranny and cruelty were soon exterminated. The appalling rapidity with which this took place may be appreciated from the estimates of the population in the succeeding years. The two or three millions of Indians present in 1492 were reduced in a period of 15 years to 60,000 by 1507, and had been further reduced to 14,000 by 1514. Enrique, the surviving caique, was so enraged by the treachery of the Spaniards, which had led to the capture and execution of Queen Anacaona, that he fled with a small band of followers to the fastnesses of the mountains, where he successfully resisted all efforts to capture him. In 1533 the remaining Indians, 600 in number, under this chief were given lands and established at Boya, a small village within five miles of the camp at which the work we are to report was done.

With the Indians practically exterminated, the land with all its wealth of resources was of little value to its new possessors. To make it again profitable, the African slave trade was instituted as early as 1508. The cruelty and abuses practiced on the Indians were repeated on the Africans, and doubtless would have been attended by the same result, but for the fact that, as they were killed or died off, fresh impor-

tations were brought in. With the decline of the Spanish power the greatest blight to the existence of the slaves was removed, and under climatic and economic conditions, which were for the most part favorable, they multiplied so that the present population of Santo Domingo consists largely of descendants of the African slaves with a greater or less admixture of Spanish blood.

Although the history of the island abounds in interest we may not attempt even a brief sketch of it in this paper. In passing one may recall that it was from Santo Domingo that Cortez, Balboa, Pizarro and De Soto set out to make their brilliant explorations and discoveries. It was the rendezvous for many of the pirate craft that preyed on the British, Dutch and French commerce with the new world. Here the term "fillibuster" originated. The long speedy boats used in this piracy were called fly-bote or freibote, and their crews were known as freiboters, freebooters or fillibusters. The British and French united to retaliate against the Spanish pirates and established a base on St. Christopher and later on the island of Tortuga, just north of Santo Domingo, where they were joined by the Dutch. In addition to their operations on the water, they made frequent incursions into the northern part of Santo Domingo for the purpose of killing the cattle. Those who engaged in this pursuit came to be known as buccaneers, from boucan, the spit on which they cooked their meat.

From Tortuga the French gained a permanent foothold on the western end of the island, the part now occupied by Haiti.

Recall that the entire island has been at one time under Spanish rule, that the Dutch and British have each gained a foothold on the island, that eventually independence from France and Spain was gained, and the Republics of Haiti and Santo Domingo were established, that each of these young republics at one time or another gained possession of the entire island, and that these various changes were not effected without blood-shed. Indeed, the history of the island from the time of its discovery by Columbus in 1492 up to its military occupation by the United States in 1916 has been one of external war and internal revolution. Even in the short history of the republics, the list of presidents is of wearying length, the common way of removing a president being by assassination, and of establishing a new government, by revolution.

The result of this constant state of turmoil was that the resources of the island remained undeveloped, the people impoverished; a relatively large national debt was acquired, lawlessness prevailed, education and sanitation were sadly neglected.

Even in the few years during which the United States Military Government has been in control, splendid progress has been made in bettering these conditions. Peace has been maintained, law and order prevail, schools have been established, agriculture has been fostered, highways (one of the greatest essentials to the development of the country) constructed, and great progress has been made in sanitation. The credit for this work is due to the splendid group of U. S. Naval officers, who have been in charge of the military

government. The customs have been collected and not only have the expenses of this work been paid for, but the national debt which amounted to approximately \$20,000,000.00 in 1907 has been reduced by about half.*

At the request of the military government of the Dominican Republic the School of Tropical Medicine, Harvard University, sent a commission consisting of Drs. A. W. Sellards, W. L. Moss and G. H. Bigelow to Santo Domingo during the summer of 1920 to study yaws. The results of the observations made are herewith presented in abstract.

CLINICAL DESCRIPTION OF YAWS

As the disease is strictly limited to tropical and subtropical countries, it may be unfamiliar to many physicians who have not been in the tropics; hence a brief introductory description of yaws is given.†

Synonyms.—Framboesia tropica, yaws, buba, pian, etc.

Definition.—A tropical specific infectious and contagious disease caused by *Treponema pertenue* Castellani and characterized by a frambesiform granulomatous eruption.

Distribution.—West Indies, Central America, northern part of South America, Africa, Malay Peninsula, Siam, Ceylon, parts of China. It is said never to occur in the mountains and cold districts and rarely above an elevation of 800 feet.

Etiology.—The *Treponema pertenue*, first observed by Castellani in 1905, is now accepted as the etiological agent of the disease. There has been much discussion concerning the question of the identity of yaws and syphilis, and while some observers still maintain their unity, the evidence at hand seems sufficient to establish them as two separate diseases. Charlouis, as early as 1882, successfully inoculated yaws in a syphilitic patient, and syphilis in a patient infected with yaws. That yaws patients are not immune against syphilis is further indicated by the observations of Powell and Nichols and of others, who have described cases of syphilis supervening on yaws.

Incubation Period.—Castellani gives the incubation period as from two to four weeks. Paulet, in 1848, inoculated 14 negroes with the secretions from frambesic granulomata, and all developed yaws in from 10 to 20 days. In monkeys and other animals, experimentally inoculated with yaws, the incubation period varied from 16 to 92 days.

Symptomatology.—The course of the disease, like that of syphilis, may be divided into a primary, secondary, and tertiary stage. Some authors add a fourth stage (paraframbesic affections), but it is questionable whether the observations on which this additional stage is postulated are reliable.

* For the history of Santo Domingo the reader is referred to two fascinating books: "Santo Domingo—A Country with a Future," by Otto Schoenrich; and "Santo Domingo, Past and Present," by Hazzard; both unfortunately out of print. Free use has been made of these and of the Encyclopedia Britannica in the brief historical note here given.

† This description is taken largely from the chapter on Framboesia Tropica, Manual of Tropical Diseases. Castellani and Chalmers, Third Edition, Wm. Wood & Co., 1920.

Primary Stage.—Incubation period two to four weeks, characterized by malaise, rheumatic pains, headache, sometimes irregular fever. The primary lesion (mother yaw, madre buba) appears as a papule, which after a week becomes moist, and develops a yellow secretion. A crust may form, which on removal reveals an ulcer with a granulomatous base. The primary lesion may heal before, but usually persists until after the secondary eruption has appeared.

Secondary Stage.—The secondary stage begins in from one to three months after the primary lesion, and is characterized by a general eruption of granulomata over the body. It is preceded by malaise, headache, severe pains in the muscles, joints and bones. In some cases there may be fever of an intermittent type. The granulomata appear first as minute papules, which increase in size to an average diameter of about one centimeter. They may coalesce, become secondarily infected, and form large ulcerating areas. In the majority of cases—within three to six months in children, and six to twelve months in adults—the granulomata dry up, shrink, and disappear. In some cases the granulomatous eruption may continue for several years, new crops of nodules appearing from time to time in succession. The granulomata generally undergo involution within from two to four months, leaving behind, as a rule, some change in pigmentation. At times secondary ulceration occurs, leading to deeper scars, and occasionally the granulomata take the form of a circinate eruption.

The granulomata frequently occur on the soles of the feet, less often on the palms of the hands, with a resulting condition closely resembling the syphilitic psoriasis palmaris and plantaris.

The alimentary, respiratory, excretory, and central nervous systems are rarely involved. The joints are frequently swollen and painful. Lesions of the mucosæ are rare. Castellani and Chalmers have not observed alopecia, but state that various groups of lymphatic glands are found enlarged. There may be a moderate degree of anæmia of secondary type and an increase in the large mononuclears. The Wassermann reaction is positive in the great majority of recent cases, but is fairly often negative in old cases.

Tertiary Stage.—The disease often terminates with the secondary stage. Sometimes the secondary and tertiary stages merge, or there may be an interval of years between them.

The characteristic lesions of the tertiary stage are gummatous-like nodules and deep ulcerative processes, frequently resulting in great deformity. The bones are quite commonly implicated, but visceral lesions are rare or absent. Castellani and Chalmers think that Gangosa, an ulcerative condition of the palate, nose, and pharynx, is in reality a tertiary manifestation of yaws.

Histopathology.—In the frambesic papules the surface epithelium is greatly thickened and numerous elongated downgrowths are seen. In patches the epithelial cells are swollen, vacuolated and degenerating. Circumscribed areas contain polymorphonuclear leucocytes. The layers near the corium are almost normal, but the corium itself is edematous. There

is a diffuse cellular infiltration consisting of polymorphonuclear leucocytes, large and small mononuclears, eosinophiles, plasma cells, mast cells, connective-tissue cells and some extravasated erythrocytes. Treponemata are present in the granulomata.

Treatment.—Brodin in 1910 tried antimony. "Castellani's Yaws Mixture" contains tartar emetic, potassium iodide, sodium salicylate, and sodium bicarbonate. Salvarsan was first used by Nichols in the experimental disease in monkeys, and by Strong in the treatment of the disease in man. The cure of yaws with salvarsan or neo-salvarsan is one of the most striking examples of specific therapy that medicine presents.

The malady does not appear to be hereditary. The course of the disease is chronic, resulting in serious disability and much suffering in a majority of cases. The mortality from yaws is not high, and death, when it occurs, is usually due to secondary infections.

THE AUTHORS' OBSERVATIONS

In discussing the cases observed by us in Santo Domingo the following abbreviations are used for convenience of reference. If the reader will take a moment to familiarize himself with these, the understanding of what follows will be facilitated:

- M. Madre buba, mother yaw, primary lesion.
- B'. Florid secondary eruption of granulomata. Early secondary stage.
- B. Sparse recurring secondary granulomata. Late secondary stage.
- C. Clavus. Late lesions on the soles. Late secondary stage.
- P. Palmar lesions. Late lesions on the palms. Late secondary stage.
- G. "Gomma." Tertiary stage.
- H. History of yaws. No active lesions present. Quiescent.

PRIMARY STAGE

We observed 64 cases in which a primary lesion was present, sometimes alone, oftener in combination with other manifestations of the disease. Briefly these cases may be presented in the following tabular form:

Diagnosis	No. of cases	Average age in yrs.	Average duration in mos.
M	15	6.6	2.3
MB'	25	8.5	7.3
MB/C	13	11.6	4.1
MB	6	24.2	19.0
MBC	5	10.	17.2

The extremes of age in this group of cases were 7 months and 60 years. The duration of the madre at the time of observation varied from one month to six years, and the size varied from 1 to 7 cm. with an average diameter of about 2.5 cm. Of the 15 cases in the above group which presented a madre buba as the only active lesion, five showed healed scars of secondary granulomata; thus there were only 10 cases which

had not progressed beyond the primary stage. In 54 cases, or 84 per cent, of our series, the madre persisted after the appearance of secondary lesions.

Location of Primary Lesion.—In 969 cases the location of the madre was determined either by history, observation of the lesion, or scar (in 635 cases, or 71 per cent, the scar was present). In 18 additional cases the patients stated that they had had no madre but that multiple granulomata had appeared simultaneously over the body. In two cases the patients claimed to have had two madres simultaneously.

TABLE SHOWING LOCATION OF MADRE

	Cases	Approx. per cent
Lower extremities	803	82.87
Upper extremities	80	8.26
Head	39	4.02
Trunk	37	3.82
Genitalia	10	1.03
	969	100

The primary lesion occurred on the right side of the body in 425 cases and on the left side in 468 cases. In the remaining cases, the site was noted as knee, ankle, etc., but the side of the body was not recorded.

These figures have been analyzed to determine if the site of the madre might throw any light on the mode of transmission of the disease. In this connection it is to be remembered that we are dealing with a bare-footed and bare-legged population, that even the adults are scantily clothed, that the upper extremities are almost as much exposed as the lower, and that children of both sexes usually wear no clothing until they are about five years of age.

Sexual Transmission.—Sexual transmission as a common method may be excluded because of the fact that in only 1 per cent of our series was the primary lesion on the genitalia. Of these 10 individuals, 6 were males, 4 females, and 5 were under 8 years of age. Moreover, in 25 per cent of all the cases in our series the infection occurred before the fifth year of life.

Hereditary Transmission.—We saw no evidence of hereditary transmission in any case.

Insect Transmission.—We think that transmission by the bites of the common flying insects, such as flies, mosquitoes, etc., may be excluded on the basis of the distribution of the primary lesion; in the great majority of cases, the madre occurred on the lower extremities, whereas the face and upper extremities are equally exposed to the bites of these insects.

The preponderance of the primary lesion on the lower extremities as compared with the trunk would likewise seem to exclude transmission by the bites of such non-flying insects as lice, fleas, bed-bugs, and the like.

The fact that in such a large majority of cases (83 per cent) the primary lesion occurred on the lower extremities led to a further analysis of these cases. The result is shown in the

following tabulation, and for comparison the figures for the upper extremities are given:

Cases		Cases	
Buttocks	13	Shoulders	6
Thighs	30	Upper arms	4
Knees	55	Elbows	16
Legs	201	Forearms	17
Ankles	258	Wrists	8
Feet	246	Hands	25
Total	803	Total	76

It will be noticed that the frequency with which the primary lesion is found on the various parts of the lower extremities increases strikingly as one approaches the ground. This suggests that if the disease is transmitted by the bites of insects, we should think of some non-flying insect which remains on or near the ground, and does not normally prey on man or even desire to domicile with him; such as ants, ground spiders, and the like.

The possibility that trauma provided the portal of entry for the treponema has been suggested. The greater frequency of the primary lesion on the exposed parts of the body fits in with such an hypothesis. It seems probable also that in a bare-footed population the lower extremities would be the most frequently traumatized part of the body, and that the frequency of minor injuries would increase as one progressed down the extremity. Examination of the preceding table shows that the frequency with which the madre occurs on the various parts of the lower extremities increases as one descends toward the foot.

Man, being a forward-going animal, is apt to get most of his knocks from in front; accordingly, we have analyzed the primary lesions occurring on the knees, legs and ankles with reference to their occurrence on the anterior, external, internal and posterior surfaces.

As far as our records showed this data, the results are set forth in the following table:

	Anterior	External	Internal	Posterior
Knees	18	8	6	4
Legs	107	35	19	12
Ankles	25	79	84	13

In the case of the knees and legs it is shown that the anterior surface leads by a large majority, the external surface comes next, followed by the internal surface, while the posterior surface finishes a poor last. This probably corresponds closely to the exposure to minor traumata.

In regard to the ankle the circumstances are different. Owing to its conformation the malleoli are about equally exposed to injury, while the anterior surface of the ankle is protected by the projecting foot which receives on its dorsum the injuries which would otherwise go to the ankle. This corresponds with the frequency of the madre on the various parts of the ankle, and with the fact that in the case of the foot, the primary lesion was found on the dorsum in a great majority of cases.

It appears probable from our observation, therefore, that trauma, frequently minor in character, usually determines the site of the primary lesion in yaws. This is entirely compatible with the view that the disease may be transmitted by direct contact, or that insects, flies, etc., may be vectors of the virus.

SECONDARY STAGE

Florid Secondary Stage.—In our series there were 144 patients who presented themselves during the florid secondary stage of the disease. In 38 of these the primary lesion was still present and in 37 the late lesions of the soles, known as clavus, had made their appearance.

Briefly summarized these cases are presented in the following table:

FLORID SECONDARY STAGE

Diagnosis	No. of cases	Avg. age	Avg. duration	No. of cases with fresh granulomata on	
MB' ...	25	8.5 yrs.	7.3 mos.	2	1
MB'C ...	13	11.6 "	4.1 "	7	1
B' ...	82	8.8 "	12.3 "	6	0
B'C ...	24	12.9 "	30.4 "	9	2
	141			24 (16.6%)	4 (2.8%)

Exclusive of the 38 still showing the primary lesion and previously analyzed, the youngest patient in this group was 1 year and the oldest 50 years.

While clavus may develop very early in the disease, witness the 13 MB'C cases with an average duration of 4.1 months, as a rule it does not develop until the disease has lasted more than a year. The average duration in the 82 patients who presented only secondary granulomata was 12.3 months, whereas the average duration of the cases which showed clavus in addition to the florid secondary eruption was 30.4 months. The greater frequency of granulomata on the soles as compared with the palms is indicated by their presence in the former situation in 16.6 per cent of this group as against 2.8 per cent in the latter situation.

From our observation and the histories we obtained, we are led to believe that, in the disease as it occurs in Santo Domingo, practically all of the patients, sooner or later, develop granulomata on the soles and become the victims of the painful condition which they designate as clavus.

The secondary eruption, as we saw it, corresponds closely to the text-book description as given by Castellani and Chalmers. The individual granuloma does not differ materially in appearance from the primary lesion except that it usually does not become so large and seems less apt to undergo as deep ulceration. In our notes we described the earliest stage observed by us, as a small papule 1 to 2 mm. in diameter, gray in color, and surrounded by a narrow zone of hyperemia. These papules increase rapidly in size to an average diameter of about 1 cm. and are usually elevated 0.5 to 0.75 cm. above the skin. The surface of the granuloma is covered with minute

bosses which give it a raspberry-like appearance from which the disease takes the name *frambesia* (Figs. 4, 5, 6, and 8). In the early stage the granulomata are of a pearly gray color; later they are apt to become covered by a thin yellow crust. After a few weeks or months retrogressive changes take place; the lesions turn dark, often black in color, shrink in size, the surface becomes checkered, and after a time all that remains is a black crust, which on falling may leave no scar; more frequently pigmentary changes persist, which may consist of increased pigmentation, apigmentation or an apigmented area surrounded by a zone of increased pigmentation.

At no stage of the granulomata was vesiculation noted. They may go through their entire existence without ulceration, but more frequently they become secondarily infected, and consequently more or less deeply ulcerated. During the florid secondary eruption there may be hundreds of granulomata over the body, and no part of the cutaneous surface, from the crown of the head to the soles of the feet, is exempt from invasion. They show a special predilection for the muco-cutaneous borders—nostrils, mouth, genitalia, anus, and moist surfaces—axillæ, internatal region, between the upper thighs and popliteal spaces (Figs. 7 and 8). The mucous membranes are said to be rarely involved and we saw but one such case in our series, a single granuloma on the vermilion surface of the lower lip. We observed no case of alopecia except as the result of ulcerative processes involving the scalp. One patient presented the typical circinate arrangement of the lesions on the face, which strikingly suggested the Indian war-paint appearance (Fig. 12).

Usually there was no complaint of pain in consequence of granulomata on the body except in cases in which they were situated on the bearing surfaces of the feet, on the palms of the hands or when extensively ulcerated.

Arthritis (Fig. 22) and dactylitis were fairly common, although they usually occurred later than the florid secondary stage of the disease. The knee-joints were involved most commonly, the elbows next in frequency. Usually a single joint was affected. The swelling was sometimes considerable and in one instance as much as 200 c.c. of slightly turbid, straw-colored fluid was aspirated from a knee-joint. This fluid was without evident effect when injected into the peritoneal cavity of a guinea-pig. The dactylitis took the form of the usual spindle-shaped fingers, generally a single digit, sometimes several, being involved.

Late Secondary Stage.—There were 190 cases in the series that were considered to be in the late secondary stage of the disease. The patients had passed through the florid secondary eruption as evidenced by the numerous scars of previous granulomata. Many of them had doubtless been entirely free from active lesions for variable lengths of time after which one or more recurrent granulomata had appeared. It is evident from the histories obtained that the disease may undergo an apparent spontaneous cure, but sooner or later granulomata reappear in a majority of cases and successive crops, consisting usually of only a few lesions, continue to make their appearance, with

free intervals between for many years. Briefly tabulated these cases may be presented as follows:

LATE SECONDARY STAGE					
Diagnosis	No. of case	Avg. age	Avg. duration	No. of cases with fresh granulomata on soles	No. of cases with fresh granulomata on palms
MB	6	24.2 yrs.	19 mos.	2	1
MBC ...	5	10.0 "	17.2 "	1	1
B	60	9.3 "	29.6 "	16	1
BC	106	16.5 "	5.4 yrs.	69	1
BCP ...	13	20.5 "	11.3 "	10	4
Total..190				98 (51.6%)	8 (4.2%)

Of the 190 patients in this group, 60 had as their only active manifestation of the disease a few secondary granulomata, 106 had granulomata plus clavus lesions and 13 had, in addition to the above, palmar lesions. Eleven cases which have already been analyzed with the madre cases are included here, as they appear to have been in a late secondary stage of the disease in spite of the persistence of the primary lesions.

The extremes of age in this group are 1 and 75 years; and while the former figure shows that a late secondary stage may be reached not only early in life but comparatively early in the disease, the average age in this group indicates that this is exceptional, and both the average age and average duration of the disease at the time of observation point to the chronicity of the secondary stage of the disease.

CLAVUS.—Perhaps the most curious and interesting effects of the disease are the late lesions which occur on the soles of the feet and it is to these that much of the pain and disability is due. We have adopted the term "Clavus" used by the natives, because it seems an appropriate one, although they use it to designate conditions of the soles long after anything suggesting a "Nail" has disappeared.

In a previous section attention has been called to the frequency with which secondary granulomata appear on the soles. Those occurring in this situation do not differ materially in appearance from granulomata seen elsewhere on the body (Fig. 13). They are perhaps more frequently secondarily infected, and are apt to ulcerate, probably as a result of the fact that the natives seldom protect them by any sort of dressing and continue to hobble about even when the feet appear to be in a shocking condition.

If the granuloma on the sole heals without ulceration, as it shrinks, it separates from the surrounding epidermis and becomes circumvallate. The hard, dry black core must act like a foreign body beneath the foot and it is the "nail" which led to the use of the term clavus. Finally, the core drops out leaving the "nail hole," a circular opening .75 to 1 cm. in diameter with sharp-cut edges which descend vertically 2 or 3 cm. to a flat base.

Usually both soles are about equally involved, although rarely cases are encountered in which only one sole is affected (Fig. 15). The number of granulomata on each sole may vary from only a few to 20 or 30. Irregular erosion, the result of attrition of the epidermis intervening between the "nail holes" may account for the remarkable moth-eaten appearance

of the soles which many of the old clavis cases present (Figs. 14, 17 and 18).

Under the designation "clavis" the natives include conditions differing from those just described and which are not so readily explained as the result of pre-existing granulomata. Not infrequently the soles presented a condition which is aptly described by the term honey-combed. The entire sole, or, in some cases only the bearing surface, was filled with pits 0.5 cm. or less in diameter, conical in shape and extending to a depth of 2 or 3 mm. These pits are as closely and regularly set as the cells of a honey-comb, and no such number or distribution of fresh granulomata was observed as would suggest that each pit could represent the site of a previous tumor.

In other cases the epidermis of the entire sole or a large part of it was greatly thickened, black, and dead-looking.

The natives applied the term clavis indiscriminately to any of the conditions just described. Pain was the characteristic common to all. Like the granulomata elsewhere on the body, the clavis lesions would clear up spontaneously in time, only to recur again and again throughout many years.

Occasionally a condition of the soles was seen to which the natives apply the name "Rajadura," which means a fissure or crack. The fissures, 2 to 3 cm. long and 2 to 3 mm. deep, are usually arranged vertically about the periphery of the heel. More rarely they occurred in the sole beneath the instep or ball of the foot, where they might attain a length of 6 or 8 cm., reaching to a considerable depth and, like those of the heel, cause much pain. We gained the impression that these fissures were also due to yaws, and for this reason have included in our analysis the relatively small number of cases encountered with clavis, although the term "Rajadura" is more descriptive.

Before proceeding to the analysis of the clavis cases it may be well to state that we have not included in this category any cases which presented fresh granulomata as the only lesions of the soles. We have reserved the term clavis to designate only the late effects of the infection on the soles, and think that these should be regarded as belonging to the late secondary stage of the disease. It may be of interest to give here a description of the changes which took place in the clavis cases following the administration of neo-salvarsan leading to their cure. A statistical statement of the result of the treatment in all cases will be given in a later section of this paper, but the results obtained by treatment furnished corroborative evidence that these several different lesions were properly considered as a manifestation of yaws.

We were prepared to expect the improvement which resulted from the use of neo-salvarsan in the cases with fresh granulomata, whether they were situated on the soles or elsewhere on the body, but it seemed rather too much to expect that these old "moth-eaten," dilapidated soles would be rejuvenated by a few injections of any drug. The first few patients were given an injection more as a placebo on account of their impotency and the long distances that some of them had come, rather than with any expectation that they would be benefited. When these patients came back at the end of a week, however, their feet

not showing the least improvement in appearance, we received with surprise and incredulity the statement that they were cured of pain and when they adduced, in support of their claim, the statement that, whereas they had been obliged to come on horse the first time, they were able to return on foot, we were encouraged to try treatment on other cases. In the end clavis comprised the largest and in certain respects the most important group of cases that were presented for treatment.

A week after the first injection of neo-salvarsan there was, as a rule, no objective change in the feet, but almost without exception the patients affirmed that the pain was either greatly ameliorated or entirely gone. The injections were usually given at from seven to ten day intervals. About a week after the second injection, the old dead epidermis loosens up about the edges of the clavis holes, erosions and fissures, and begins to desquamate. This process goes forward rapidly until within a week or two after the second injection all the old epidermis has been shed, exposing healthy pink skin.

The following table summarizes the clavis cases:

Diagnosis	No. of Cases	Avg. Age	Avg. duration	Sole affected		
				Right	Left	Both
MB'C	13	11.6 yrs.	4.1 mos.	8	11	6
MBC	5	10. "	17.2 "	4	4	3
B'C	24	12.9 "	30.4 "	21	23	20
BC	106	16.5 "	5.4 yrs.	94	92	80
BCP	13	20.5 "	11.3 "	11	13	11
C	327	21.6 "	9. "	299	291	266
C+*	41	29.4 "	12.9 "	33	35	27
CP	50	32.7 "	18.7 "	45	47	42
	579			515	516	455

In 3 of the 579 clavis cases no note was made as to which sole was involved. It is apparent, however, that the right and the left sole are affected with equal frequency, and that in a majority of cases both soles are implicated (79 per cent in the above series).

Although we have seen clavis in an infant of one year, attention is called to the fact that the average age of the largest group in this series, the 327 patients who had clavis only, was 21.6 years, and that the average duration † of the disease in this group at the time they presented themselves for treatment was 9 years.

Palmar Lesions.—Perhaps corresponding* to the clavis lesions, though not so definitely dependent upon granulomata, are the painful keratoses and fissures which, late in the disease, appear on the palms of the hands. There is sometimes more or less erosion of the epidermis and frequently an inability to open the hand fully and extend the fingers. This is apparently due to a certain amount of contraction of the hard, dry keratosed surface.

* The group of 41 cases designated as C+ had in addition to clavis some other condition which apparently was not dependent on the yaws infection. In a majority of cases the additional affection consisted of chronic leg ulcers.

† Wherever average duration is given in this paper it refers to the duration since the first appearance of the primary lesion and not to the duration of any particular stage, unless so specified.

The condition is usually painful, though less so than clavus, and interferes with manual labor. It is a much rarer lesion than clavus, and appears to develop in older individuals and later in the disease, though it seems to be equally amenable to treatment.

In our series this condition was met with 68 times, but only five of these patients had palmar lesions (exclusive of scars) as the only effect present. These 68 cases are briefly summarized in the accompanying table:

Diagnosis	No. of cases	Average age	Average duration
BCP	13	20.5 yrs.	11.3 yrs.
CP	50	32.7 "	18.7 "
B	5	25. "	14. "

It is rather striking that of the cases presenting a single manifestation of yaws, clavus forms the largest group, 327 cases, while those showing palmar lesions (other than fresh granulomata) constitute the smallest group—five cases. It is also striking in the above summary of the palmar cases, that those associated with clavus as the only other lesion form the largest number—50 out of 68 cases.

It is interesting that the average duration of the disease in the CP group, 18.7 years, is the longest of any group of the entire series, not excepting those in the tertiary stage of the disease.

Studded Lesions.—In a number of cases we observed nodular skin lesions which we believe to be a manifestation of yaws (Fig. 20). The earliest stage of these nodules was either not observed or else was not recognized as the beginning of lesions which in a later stage of their development became quite familiar to us. Apparently, these lesions may occur on any part of the body, although we never observed them on the head or face. The forearms and legs were the commonest sites in the cases which we observed. The thighs and trunk were frequently implicated and lesions starting on the dorsum of the foot and extending on to the plantar surface were noted.

A typical picture of the condition as observed by us may be described as follows: The individual lesion consists of a moderately hard skin nodule, 1 cm. in diameter, elevated 3 to 4 mm., not painful, unaccompanied by itching and without striking pigmentary changes until after regression, when increase of pigment may mark its former site. These nodules are thickly studded and regularly set over an area 8 to 10 cm. in diameter. The size of the area involved increases by an advancing margin consisting of an almost unbroken row of nodules. As this peripheral advance occurs, healing takes place in the center of the area. If the nodules do not ulcerate this retrogression is accompanied by desquamation of the epithelium. The nodules gradually flatten out and finally disappear, leaving no trace, or more often a circular area of increased pigmentation.

In no case were the nodules observed to pass through a vesicular stage, but they frequently underwent ulcerative changes varying from very superficial ulceration involving only the individual nodules to extensive and deep ulceration which sometimes became confluent over large areas of the body and

extended to the subcutaneous tissues. The degree and character of the resultant scarring probably depends upon the depth to which the ulceration had extended. Sometimes there remains only an increase of pigmentation, as mentioned above; sometimes the skin over the entire area involved was left thin and crinkly and in patterns like those which form on the surface of hot chocolate which has been thoroughly boiled and allowed to stand awhile. Again, where the ulceration has extended to a greater depth there may result complete leucoderma and sometimes painful keloid (Fig. 21).

We have called these lesions "studded" for the lack of a more descriptive term. The area involved, when small, is usually circular, but as it increases in size it is apt to become elliptical or more often it approaches a rectangular shape but with rounded corners. If situated on an extremity, the long diameter of the area coincides with the axis of the limb. Seldom does it completely encircle an extremity even when large areas are involved.

We regret that we had no opportunity of studying these lesions histologically. They do not in the least resemble the primary and secondary granulomata. We do not know if they should be considered a late secondary manifestation of the disease or if they belong to the tertiary stage.

We have notes on 37 cases, the youngest patient exhibiting these lesions being 5 years of age and the oldest 80 years. The average age in this group was 28.8 years, which comes next in length to the gamma group (29.3 years). The average duration of the disease (yaws) in this group was 13.5 years with the extremes of duration 5 months and 39 years. This average duration is exceeded only by the P, G, and CP groups.

Our belief that these skin lesions are a manifestation of yaws is based on the fact that they were observed only in patients who gave a history of yaws, or who had other recognized yaws lesions, and on the observation that they apparently responded specifically to neo-salvarsan therapy.

We are inclined to regard them as a late secondary manifestation of the disease.

TERTIARY STAGE

The impression gained from a fairly close study of over a thousand cases leaves no doubt in our minds that yaws and syphilis are separate diseases. Exclusive of the evidence contained in the literature of inoculation experiments, the occurrence of yaws and syphilis in the same patient, the difference in the response of the two diseases to mercury, the possibility of reinoculation in yaws, the localization of the disease to the tropics and below certain altitudes, the grounds afforded by our own experience on which we base our opinion are as follows: Syphilis is universally recognized as a venereal disease, the primary lesion is, in the vast majority of cases, located on the genitalia and even in the cases not contracted through sexual intercourse, in which the primary lesion is extra-genital, the course of the disease is in no way modified by the method of inoculation or the unusual seat of the chancre. Yaws, as we have already pointed out, can be excluded from the category of venereal diseases by the fact that in a series of approximately

a thousand cases the primary lesion was located on the genitalia in only 1 per cent and infection took place in 25 per cent before the fifth year of life. Moreover, the primary lesion in yaws differs distinctly in appearance from chancre, even when the latter is extragenitally located, the madre buba, as a rule, being larger, the surface rougher; moreover, in no case was there the hardness sometimes present and usually considered characteristic of the syphilitic lesion.

In no case did we observe any evidence or obtain any history of hereditary transmission of the disease.

The duration of the madre buba and the high percentage of cases in which it persists after the onset of the secondary stage of the disease (84 per cent in our group of 64 madre cases) is contrary to what is observed in syphilis.

When we come to consider the secondary stages of the two diseases we find more striking contrasts and more especially do we note the remarkable pleomorphism of the secondary lesions of syphilis and the faithful monotony of the secondary granulomata of yaws. The versatile *Spirocheta pallida* may occasionally produce a counterfeit yaws but never did we observe a yaws patient in the secondary stage of the disease who failed to show either typical granulomata or scars which we felt sure were the marks of typical granulomata. Even the secondary infections and ulcerations which may result do not disguise the nature of the lesions.

Although Castellani and Chalmers say that "occasionally, peeling, whitish patches may be seen on the palms of the hands and soles of the feet closely resembling the syphilitic psoriasis palmaris and plantaris" we have never observed in syphilis anything resembling the clavus and palmar lesions which we have described as occurring in yaws. On the other hand, none of the yaws cases which we observed presented such characteristic secondary manifestations of syphilis as the macular skin rash, mucous patches and alopecia.

In the tertiary stage of the disease the absence of visceral involvement especially of the heart, blood vessels, liver and kidneys is in striking contrast with the frequency of their involvement in syphilis. Moreover, in a series of over a thousand cases in which the average duration for the entire series was 20.3 years and 79 of which were in the tertiary stage, none showed any evidence of central nervous system involvement. We do not wish to attach undue significance to the absence of central nervous system lesions in this series of cases because the relative immunity of the colored race to such involvement in syphilis is well known.

Finally, the marked difference in the response of yaws and syphilis to neo-salvarsan is a not unimportant point in favor of the duality of the two diseases. We have seen the lesions of a patient in the florid secondary stage of the disease melt away completely after a single injection of 0.6 gm. of neo-salvarsan. Indeed the cure of yaws by this agent is the most dramatic therapeutic performance that we have ever witnessed.

But although a decision between syphilis and yaws in the primary and secondary stages was arrived at with little difficulty, there were cases presenting tertiary lesion in which we found it impossible to make the differential diagnosis (Figs.

23, 24, and 29). There were patients with periosteal thickenings of the long bones; especially of the tibia, radius and ulna. There were those with typical spindle-shaped swellings of one or more fingers, and others with the bridge of the nose destroyed and with perforation of the hard and soft palate (the condition known as gangosa). There were cases in which old ulcerative processes had led to loss of substance of the cranial and other bones. Some of the patients who had an eye that had ulcerated out, and some in whom the nose was almost completely destroyed or fingers or toes had been lost, suggested the ravage of leprosy. Whether these results were due to syphilis or yaws we are unable to state positively. The patients themselves applied the term "Gomma" to all tertiary lesions and stoutly maintained that they were due to the latter disease "yaws."

There were other patients exhibiting tertiary manifestations which bore no resemblance to syphilis as we see it in the United States to-day. These included extensive skin ulcerations, great enlargement and deformity of the hands and especially of the legs and feet.

As previously mentioned, pain in the joints and often an effusion was a frequent complaint in the secondary stage. Many of the tertiary cases showed arthritic enlargement and partial or complete fixation.

That those cases, objectively indistinguishable from syphilis, were actually yaws, we must, of course, leave unsettled. In favor of the view that they were yaws are the following facts:

The patients themselves, who are familiar with both diseases, maintained that the condition from which they were suffering was yaws. Practically all of this group denied having had syphilis (this denial deserves some consideration, because little or no shame attaches to venereal infection among the natives). There were 79 individuals in the "Gomma" group and of these 67 were able to give the location of the primary lesions. Corroborative evidence of the correctness of their statements as to the location of the primary lesion was furnished by a scar in 34 cases. In only two of the 67 cases was the location of the primary sore at all a usual one for a chancre, the upper lip in one case and perineum in the other.

SUMMARY OF THE GOMMA CASES

Diagnosis	No. of cases	Average age in years	Extremes	Average	Duration in years Extremes
G	67	29.3	3-85	16.3	7/12-59
MG	1	25.2	7-60	9.7	1-40
BG	3				
BCG	2				
CG	5				
CPG	1				

The youngest patient in the entire gomma group was 3 years, the oldest 85 years. The average age for the entire group, as well as the duration of the disease at the time of observation, was greater, as would be expected, than in any other group with the exception of the cases with palmar lesions. The extremes both of age and duration are strikingly far apart.

It is of interest that in one case the primary lesion persisted after tertiary manifestations had made their appearance.

That the disease may progress to the tertiary stage in a comparatively short time is shown by the fact that in 10, or 7.9 per cent, of the gamma cases the disease had existed 2 years or less at the time of observation.

LATENT YAWS

We have included in our series 127 individuals who had no active lesions of yaws at the time they were observed but who gave a history of having had the disease and who, in most cases, exhibited confirmatory evidence in the way of scars. Brief histories were taken and examinations made of all these cases, inasmuch as they furnished certain statistical data of some interest. Moreover, being impressed with the chronicity of the disease, the frequent recurrence of active lesions after longer or shorter periods of latency, with the doubt in our minds that spontaneous cure occurred in any considerable proportion of the cases, and at the earnest solicitation of these individuals many of whom would say "I had clavus last month and I will have it again next month" or "I have a breaking out of the buba every year," we made a practice of giving one or more injections of neo-salvarsan to patients in whose cases the history and examination satisfied us that they probably had had yaws.

The 127 cases which come under this heading may be tabulated as follows:

Diagnosis	No. of cases	Age in years		Duration in years	
		Average	Extremes	Average	Extremes
H	55	21.2	1-80	8.8 yrs.	2/12-50
H+	27	22.9	2-62	10.2 "	6/12-45
H ulceration ..	45	27.6	10-80	10.6 "	3/12-59

Of the cases in this group those designated H were without active lesions of any disease, but gave a satisfactory history of having had yaws. Under H+ we have grouped those individuals who, in addition to giving a history of having had yaws, had symptoms or signs of some other disease such as various skin eruptions, pains in various parts of the body, extensive scarring and other manifestations. One man gave a history of chancre and had a scar on the prepuce. The H ulceration group comprise 45 cases which in addition to a history of yaws showed active ulceration on some part of the body, usually old leg ulcers, which we could not definitely identify as yaws, and in most cases believed to be due to some other condition.

It seems not unlikely that this group of 127 individuals must have included many sufferers from latent yaws who, had they been left untreated, sooner or later would have again developed active lesions and not only suffered the disability arising from the disease themselves, but would also have become active agents in the spread of the disease. The statistics of this group seem to lend weight to this view. The average age in this group (23.8 yrs.) is lower than the average age of various other groups which showed active lesions, *e. g.*, groups MB, C+, CP, P, G, and G+. Moreover, the average duration in this group (9.7 years) is shorter than that in several groups showing active lesions, *e. g.*, C+, CP, P and G.

Indeed in 33, or 26 per cent, of these 127 cases the primary lesion had developed within 2 years prior to the time they applied for treatment. It seems highly probable that some of these 33 patients may have been in a free interval between the primary and secondary stages of the disease and that the majority of them were in a latent period following the early secondary eruption.

With a very limited time in which to work among a given population and with the object of accomplishing as much as possible toward eradicating the disease from the locality in which we were stationed, we think the policy of treating patients who gave a history of having had the disease, even though without active lesions at the time, is fully justified.

GENERAL REMARKS

The *epitrochlear glands* were noted in 886 cases and were found to be palpably enlarged in 519, or 58.5 per cent; they varied in size from a grain of wheat to 1 or 2 cm. in diameter. Sometimes two or three glands, 1 cm. in diameter, were found in the epitrochlear region.

The *femoral glands* were noted in 121 cases and found enlarged in 100 per cent of the cases noted. In a majority of cases the femoral glands formed a visible ovoid swelling in Scarpa's triangle frequently attaining a width of 3 or 4 cm. and a length of 5 or 6 cm. It seems probable that the enlargement of this group of glands may have been due to the frequent pyogenic infections on the feet and legs that occur in a bare-foot population.

The *spleen* was noted in 216 cases and found enlarged in 58, or 26.8 per cent. Malaria is common among the natives and may account for the large percentage of cases with splenic tumor.

The *Wassermann test* was made in 91 cases and found strongly positive in 78 cases (85.7 per cent), moderately positive in 4 cases, weakly positive in 1 case and negative in 8 cases.

The 8 cases with negative Wassermann reactions were distributed with reference to diagnosis as follows:

2 cases diagnosed	B
1 " "	C+
1 " "	SL
1 " "	H
1 " "	H+
1 " "	G
1 " "	G+

The Wassermann test was repeated in a few cases after one or two treatments, the interval between the two tests being not over a few weeks. In no case did the result of the second test differ from that of the first. If it had been possible to perform the test at a longer interval after treatment, the results might have been different.

Thus it will be seen that in the small number of cases in which we have data on the Wassermann reaction the negative reactions all occurred in the late secondary stage, the tertiary stage, or in patients who had no active lesions but who gave a history of yaws.

SEQUENCE AND DURATION OF THE VARIOUS STAGES OF YAWS

In the discussion of this entire series of cases we have tried to take up the various distinctive manifestations of the disease in the order of their development. In so chronic a disease as yaws it was obviously impossible, in the limited time at our disposal, to observe a single case from the appearance of the madre buba, through the various stages, to the final deformity produced by the gummatous lesions. Moreover, it is apparent from the preceding sections that there is much overlapping of the various stages, many patients presenting two or even three different manifestations of the disease at the same time. In one case the madre buba persisted after the tertiary lesions had developed. Indeed, not being able to observe the sequence of events, we were often ignorant as to what it had been. We believe, however, that a statistical study of the data collected enables us to unravel this tangle. Accordingly, we have tabulated those cases that presented only a single manifestation of the disease, arranging them according to the average age of each group. The table also shows the extremes of age as well as the average and extremes of duration for each group.

Diagnosis	No. of cases	Age		Duration	
		Average	Extremes	Average	Extremes
M	15	6.6 yrs.	9/12-12 yrs.	2.3 mos.	1/12-7/12 yrs.
B'	82	8.8 "	1 1/2-50 "	12.3 "	1/12-7 "
B	60	9.3 "	1-42 "	29.6 "	4/12-17 "
C	327	21.6 "	3-68 "	9 yrs.	4/12-58 "
H	127	23.8 "	1-80 "	9.7 "	2/12-59 "
P	5	25 "	15-45 "	14 "	10-19 "
SL	37	28.8 "	5-80 "	13.5 "	5/12-39 "
G	67	29.3 "	3-85 "	16.3 "	7/12-59 "

720

This series comprises 720 cases and the number included in a majority of the groups is large enough to give averages that are probably fairly reliable. It seems to us that this arrangement according to average ages probably indicates the sequence in which the various lesions develop. Confirmatory evidence in support of the correctness of this view is furnished by the fact that the average duration of the disease, at the time of observation, in the various groups, with one exception (Studded Lesions) follows the same orderly progression as do the ages. The fact that the extremes of age and duration in each group may vary widely does not, in our opinion, invalidate the deduction which we have just made.

We have attempted to use the data contained in the above table to answer certain other questions. It will be noted that the average ages and average durations are given as of the date of observation, and in the case of duration this does not represent the actual average duration of the disease or any particular stage of it. To illustrate: A patient in the primary stage of the disease may have given a history of having had the madre buba 2 months at the time he applied for treatment; it is quite possible that this lesion would, without interference, have persisted for another 2 months. Thus in that particular case the duration of the primary lesion would have been 4 months. If our reasoning is correct, the actual average duration of the madre buba may be estimated by determining

the average duration of the lesions at the time of observation in a sufficiently large number of unselected cases under natural conditions and multiplying this average by two. Thus, in our series of madre cases, the average duration at the time of observation was 2.3 months, and if 15 cases were a sufficiently large number for generalization, we could say that the actual average duration of the madre stage of yaws is 4.6 months.

Furthermore, if one stage supervened promptly on the termination of the preceding stage, we could in turn calculate the actual average duration of each of the manifestations in the above series. For example, we would arrive at the average duration of the B' lesions by multiplying 12.3 months by 2 and subtracting the average duration of the madre stage. Thus we would find 20 months as the average duration of the florid secondary stage of the disease. The difficulty about applying such simple mathematical calculations to a disease like yaws is that there may be an interval free from active lesions and symptoms between the various stages of the disease, on the one hand, and, on the other hand, a considerable overlapping of the stages. This difficulty could have been overcome if it had been possible for us to obtain accurate histories of the date of onset of each stage of the disease. This we did not attempt, owing to the ignorance of a majority of the patients and the limited time at our disposal.

Although we obtained a history of the primary lesion persisting for 6 years in one case and saw patients with a florid secondary eruption, in one case 3 years, in another 7 years and in still another case 19 years after the onset of the disease, if we may accept the history given by these patients, it seems probable that the average duration of the florid secondary eruption is less than 2 years and that the late secondary lesions, especially clavus, may recur throughout a long period which, while frequently much longer, averages about 16 years and that in some cases patients may live to extreme old age, 85 years in one case in our series, with tertiary lesions.

RESULT OF TREATMENT

Neo-salvarsan was used in the treatment of the cases here reported. The drug was dissolved in freshly distilled water in the proportion of 0.1 gm. to 2 c. c. and injected within 30 to 45 minutes after being put in solution. The intravenous method was used in all cases except for young children with v ins difficult of access, these patients receiving the injections intra-muscularly in the buttock. The dose varied from 0.075 gr. for an infant under 1 year of age to 0.6 gm. for a fully developed adult. Intermediate doses were given in proportion to age and body weight. While reactions were frequent, including chill, fever, headache and general malaise, we observed no permanent ill effects and in none of the patients who received the intramuscular injections did we observe abscess formation which reached the surface, although some of them showed a painful brawny swelling.

It should be remembered that we were working under field conditions with no facilities for hospitalizing patients even over night, that many patients came two, three, and even four days journey on horse or on foot, over difficult trails, beaten



Fig. 1



Fig. 2



Fig. 3

Primary Stage. Madre Buba or Mother Yaw.

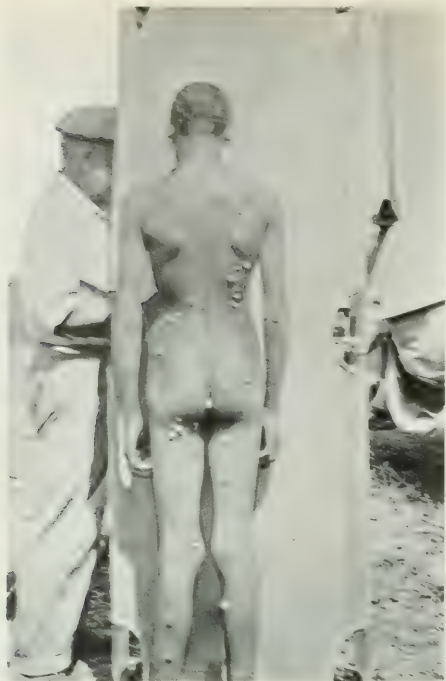


Fig. 4

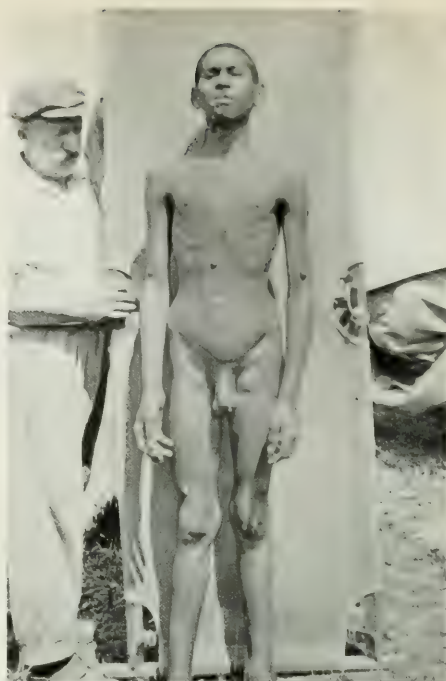


Fig. 5



Fig. 6



Fig. 7



Fig. 8

Secondary Stage. Figs. 4, 5, 6 and 8, Florid Secondary Eruption. Note Swelling of the Femoral Lymph-Glands in Fig. 5 and the Predilection for the Muco-Cutaneous Borders and Moist Surfaces in Figs. 7 and 8.



Fig 9



Fig 10



Fig 11



Fig 12

Secondary Stage. Figs. 9 and 10 show Granulomata of Unusual Size. Fig. 12 shows Circinate Arrangement of Lesions on Face, Giving the Indian War Paint Appearance.



Fig 13



Fig 14



Fig 15



Fig 16



Fig 17



Fig 18

Late Secondary Stage. Figs. 13, 14, 15, 17 and 18 Clavus. Note the Fresh Granulomata on the Soles in Fig. 13, the Unilateral Involvement in Fig. 15, and the Marked Erosion in the Remaining Cases. Fig. 16 shows Marked Thickening of the Skin and Subcutaneous Tissues of the Feet, Probably a Rare, Late Manifestation of Yaws.



Fig 19



Fig 20



Fig 21



Fig 22

Fig. 19. -A "Yaws" Family. Fig. 20. "Studded Lesions." Fig. 21. Leucoderma Following "Studded Lesions." Fig. 22. Arthritis, Late Secondary Stage.



Fig 23



Fig 24



Fig 25



Fig 26

Tertiary Stage. "Gomma." Figs. 23 and 24. Indistinguishable Clinically from Syphilis.



Fig 27



Fig 28

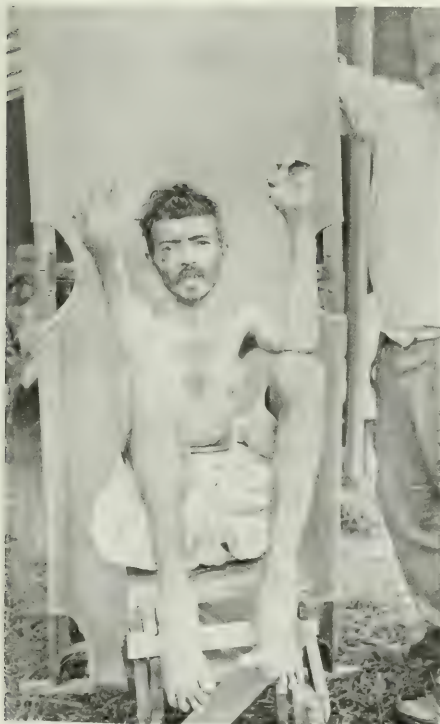


Fig 29



Fig 30

Tertiary Stage. Fig. 28.—Bears Some Resemblance to Leprosy. Fig. 29.—Indistinguishable Clinically from Syphilis.



at the time of this note. The 127 patients who gave a history of having had yaws but who had no active lesions of the disease at the time they presented themselves for treatment are not included in this table, as we had no means of judging of the results of treatment in these cases. We have also excluded from the table 31 miscellaneous cases of yaws in which the results of treatment were difficult to determine owing to complicating diseases or for other reasons. Exclusive of these two groups there are 888 cases and in 570 of these we have notes as to the result of treatment.

Briefly summarized, irrespective of the number of injections each patient received, this table shows the following results:

	Cases	Approx.
Cured	93	16.32
Practically cured	83	14.56
Much improved	179	31.41
Improved	126	22.10
Unimproved	89	15.61
Total	570	100

There were 362 cases in the above series in which the final result noted was after a single injection of neo-salvarsan. Briefly summarized they are shown in the following table:

RESULTS AFTER A SINGLE INJECTION OF NEO-SALVARSAN

	Cases	Per cent
Cured	29	8.01
Practically cured	43	11.88
Much improved	113	31.21
Improved	99	27.34
Unimproved	78	21.54
Total	362	99.98

Perhaps one gets a better idea of the efficacy of treatment in yaws by considering the cases which showed no improvement following the administration of neo-salvarsan. In the above series of 570 cases only one patient (a gamma case) showed no improvement after three injections and only ten patients (3 gamma and 7 clavus cases) showed no improvement after two injections. This is certainly in marked contrast to the 113 cases which showed marked improvement after a single injection.

We have further analyzed our figures to see if they will show what stage of the disease is most readily amenable to treatment. For this purpose it seems probable that more reliable information will be obtained by combining the cured and

practically cured groups and considering only those cases in which the result noted was obtained after a single injection. They are summarized in the following table:

Diagnosis	Total No. of cases	Average age in years	No. of cases Results noted	Cured or practically cured after one injection Number	Per cent
M	15	6.6	11	7	63.63
MB'	25	8.5	15	6	40.00
MB'C	13	11.6	11	2	18.18
MB	6	24.2	3	0	
MBC	5	10.0	2	0	
B'	82	8.8	66	27	40.91
B'C	24	12.9	20	7	35.00
B	60	9.3	36	5	13.88
BC	106	16.5	80	7	8.75
BCP	13	20.5	6	0	
C	327	21.6	184	8	4.34
C+	41	29.4	28	1	3.57
CP	50	32.7	35	0	
P	5	25.0	2	0	
SL	37	28.8	20	2	10.00
G	67	29.3	43	0	
G+	12	25.2	8	0	

The number of cases comprising some of the groups in the above table is too small to be of value for statistical purposes, but taken as a whole the figures seem to show what we would expect, *i. e.*, the earlier the stage, the more readily does it respond to treatment.

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STUDIES ON A CASE OF CHROMIC ACID NEPHRITIS

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A great deal of light has been shed upon the obscure points of kidney pathology by the newer studies on experimental nephritis. These observations have not only clarified many of our ideas about diseased kidney function, but have also contributed much to a clearer understanding of the complex and highly specialized functions of the kidneys in health.

In the production of experimental nephritis, chemical substances have been employed almost exclusively; arsenic, cantharidin, uranium nitrate, potassium bichromate, the tartrates and mercuric chloride being most frequently used. As the result of these studies we know that in a general way arsenic and cantharidin are glomerular poisons, while uranium, potassium bichromate, and the tartrates and mercury seem to have an especial affinity for the tubules. The more recent work, particularly that of MacNider,¹⁰ has shown that, although a hard and fast line cannot be drawn, yet we are still justified on the basis of outspoken affinity in speaking of glomerular poisons and of tubular poisons.

The study of cases of poisoning from these chemicals that come into the hospital for treatment is important because of their relation to experimental nephritis. Corrosive sublimate has furnished the largest number of cases in this group. Lewis and Rivers⁸ have published extensive chemical studies on a case of bichloride poisoning and have pointed out that, while the therapeutics of this condition have merited much attention in the literature, studies on the metabolism of these cases have been very meager and superficial. The number of other published cases of chemical nephroses is extremely small.

The following studies on a case of chromic acid poisoning are presented as a contribution to the subject of kidney pathology and particularly for comparison with the numerous observations in the literature on experimental chromate nephritis. This patient, suffering from an inoperable carcinoma of the face which had been treated by the application of chromic acid crystals, lived for four weeks after the development of the nephritis. During this period extensive studies of his blood and urine chemistry were carried out, marked variations from the normal were encountered and certain interesting changes brought about by therapeutic measures were noted. These observations have a certain interest as a study of the acidosis accompanying chromate nephritis and as a commentary on theories of renal secretion, particularly the "modern theory" of Cushny.²

Chromic acid was used as a cauterant as long ago as 1845 when Alexander Ure³ described several cases of hemorrhoids treated with this agent. Marshall¹² in 1857 reported a series of cases of venereal warts in which the local application of an aqueous solution of chromic acid produced excellent results. Many similar reports appear in the literature during the succeeding years, although Gubler⁴ in 1821 stated that "the

absorption of chromic acid is not free from danger, as patients have been poisoned by a too extensive application to the surfaces of their bodies." J. William White²⁴ in 1889 reported the case of a young woman who died 27 hours after the local application of an aqueous solution of chromic acid to an extensive crop of venereal warts.

Industrial poisoning among workers in chrome plants from chromic acid has long been recognized. Pye,²⁵ in 1885, reported such cases and described the perforation of the nasal septum long known among workers as "chrome holes." He stated that the possession of such "chrome holes" is an object of pride to the workers, who often carry in their pockets a bent wire which they pass through the hole for the amusement of their friends.

Gergens,² in 1877, studied the toxic effect of chromic acid on rabbits and noted that it produced an acute nephritis. Kabierske,⁷ in 1880, studied the pathological changes produced in the kidney by this chemical and Posner¹¹ continued and amplified these studies. Both Kabierske and Posner described in detail the exquisite tubular nephritis also. Kossa⁹ observed that potassium chromate produced a hemorrhagic nephritis, but was interested particularly in the accompanying glycosuria which he described as "chromic acid diabetes." Viron²³ noted the frequent occurrence of both glycosuria and albuminuria in animals poisoned with chromate, but did not carry out any extensive pathological studies. Among later investigators who used the chromates for the purpose of studying experimental nephritis are Schlayer and Hedinger,²⁰ Heinike and Meyerstein,⁶ Ophüls,¹⁹ Hellin and Spiro,¹ Pearce Hill and Eisenbrey,¹³ Weber,²² Ruschaupt,¹⁸ Austin and Eisenbrey¹ and MacNider.¹⁰ These observers noted its particular though not exclusive affinity for the renal epithelium, and MacNider states that in the earliest stage there is a vascular injury followed rapidly by a tubular involvement which soon dominates the picture.

The patient upon whom these observations were made was a man of 59 and was suffering from a very extensive carcinoma of the left cheek which had been present for two years. This lesion, when first seen, involved almost the entire cheek, extending from the inner canthus of the left eye down upon the left half of the nose and thence down and over the cheek. The patient's mother had died of chronic nephritis, one brother had died of acute nephritis and another brother still living was suffering from chronic nephritis. The patient's urine was examined for the first time on December 8, 1920, and was normal in every respect, as was also a specimen examined on December 11, 1920. On December 11 the carcinoma was curetted and crystals of chromic acid applied until an eschar had formed. The urine, when examined on the following day, showed 4 gm. of albumin per liter. The amount of albumin

in the urine increased and three days later there were 7.3 gm. per liter, and hyaline and granular casts, red blood cells and numerous degenerated epithelial cells made their appearance.

On December 14, a little more than 48 hours following the application of the chromic acid crystals, the patient had an almost total suppression of urine and the blood urea was 60 mg. per 100 c. c. From this time on he waged an unsuccessful fight which terminated with his death on January 9, his blood urea on the day of his death reaching 340 mg. per 100 c. c. Many marked changes in his blood chemistry and in the excretion of fluids and solids took place. An intravenous phenolsulphonaphthalein test was carried out on six occasions. On December 17, December 18, December 23 and on January 8 none was excreted, on December 27 and 31 the urine showed a faint unreadable trace. One gram of potassium iodide taken by mouth on January 30 was excreted in 24 hours. The McLean index which was determined every day varied from 0.01 to 0.47, the lowest values being at the onset of the nephritis and again towards the end.

A very marked feature of this case was the comparative well-being of the patient for more than two weeks following the appearance of the nephritis. During this period of 18 days he felt comparatively well except for some loss of appetite and occasional headaches, in spite of the fact that his kidneys were very severely damaged, his excretion of solids markedly diminished, and his blood urea soaring up around values ten times that of the normal high average. He was very impatient of the therapeutic measures, which consisted of forced fluid by mouth, saline purges, sodium bicarbonate administered by mouth, and rectal saline irrigations.

On December 28 his pulse became somewhat irregular and on December 31, 19 days after the onset of the nephritis, vomiting appeared which became increasingly frequent and was especially severe the last five days. His urinary output remained at a high level but sank very low the last five days, apparently because of the markedly lowered fluid intake. The patient never showed the slightest symptoms of uremia, no visible edema developed and the blood pressure varied from 145 to 110 systolic and 90 to 75 diastolic. He was given glucose intravenously, caffeine subcutaneously but gradually became weaker and weaker, the cardiac arrhythmia became more marked and he died on January 10, 30 days after the application of the chromic acid crystals.

At autopsy only the kidneys were removed. They measured $12 \times 5 \times 4.5$ cm., the right kidney weighed 176 gm., the left 197 gm.; both were mottled in appearance, the cortex was swollen and the glomeruli were very indistinct. There was no free fluid in the abdominal cavity and no general anasarca.

Microscopically the kidneys showed extensive destruction of the tubular epithelium with debris in the lumen and marked swelling of many intact epithelial cells, the changes being especially marked in the convoluted tubules. There were a few mitotic figures seen in these cells, although the evidences of regeneration were not striking. Similar observations in experimental chromate nephritis were made by Kabierske (l. c.) and by many later investigators. The glomeruli were

for the most part normal in appearance, although in some sections a few fibrosed glomeruli were seen. There were numerous areas of small-round-cell infiltration and much fibrosis in many places between the tubules. Because of the patient's age, it is doubtful just how much of the glomerular and interstitial fibrosis was due to the chromate. In general, the picture was that of a pure tubular nephritis.

A great deal of care was exercised in obtaining specimens and there is every reason to believe that no gross errors occurred that would materially affect the calculations. As the chemical studies were quite extensive, it is simpler to discuss each group separately.

METHODS

The non-proteid nitrogen, creatinin, creatin, uric acid and sugar determinations in the blood were carried out by the method of Folin and Wu. The blood urea was estimated by the method of Van Slyke and Cullen, blood amino acids by the method of Van Slyke, blood chlorides by the method of McLean, Van Slyke and Donleavy, blood calcium by the method of Halverson and Bergheim, phosphates by the method of Bloor.

The urine chlorides were determined by the method of Volhard and Arnold, the total nitrogen by the method of Folin and Farmer; creatinin, creatin and uric acid by the methods of Folin, Benedict and Myers and of Folin, and the urea by the method of Van Slyke and Cullen.

1. FLUID EXCHANGE

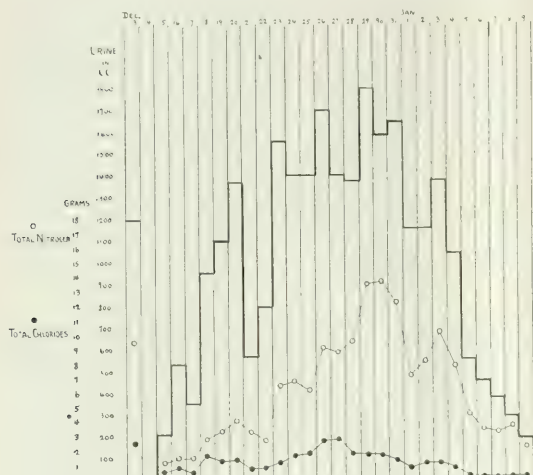
The observations on the fluid intake and output in this patient were quite complete and cover the entire of his stay in the hospital. The patient was urged at the onset of the nephritis to drink large amounts of water, which he did until a few days before death. No initial polyuria was observed such as has been described in the vascular reactions due to glomerular poisons. On the second day following the development of the nephritis, the urinary output was markedly reduced, falling to 10 c. c. Following the drinking of large amounts of water, the urinary output the following day again rose to 310 c. c., and there was a constant increase until the sixth day after the partial suppression. The total amount of urine excreted was 1360 c. c. During the period of lowered urinary excretion, the urine contained large amounts of albumin with numerous epithelial cells and epithelial casts—evidence of extensive tubular destruction. This suggests that the swelling of the tubular epithelium and choking of the tubules with debris may have caused the reduced urinary output, as observed by MacNider in experimental nephritis.

The urinary secretion remained uniformly high until four days before the death of the patient when it fell sharply reaching 280 c. c. the day before the patient died, this reduction corresponding in time to the period of continual vomiting with a marked diminution of fluid intake. The urinary record did not represent the entire fluid output, since the patient was purged constantly and considerable amounts of fluid were passed in this way. The specific gravity of the urine which

was 1.025 the day the nephritis appeared, afterward varied from 1.011 to 1.019, the average specific gravity, as determined by adding all the observations together and using their number as a divisor, being 1.014. The reaction of the urine was acid before any therapy was instituted, but following the administration of sodium bicarbonate became alkaline, becoming again acid whenever this therapy was discontinued.

2. TOTAL NITROGEN AND CHLORIDE EXCRETION

The total nitrogen, non-proteid nitrogen and chloride excretion fell very sharply following the onset of nephritis reaching the low level of 1.17 gm., .96 gm. and .59 gm., respectively. This marked depression persisted for four days and then was followed by a slow and gradual rise, until ten days after this depression the patient excreted 13.5 gm. of total nitrogen,



CURVE 1.

13.4 gm. of nonprotein nitrogen and 1.62 gm. of chlorides in 24 hours. Similar curves of nitrogen excretion were observed by Pearce, Hill and Eisenbrey, by Green, and by Austin and Eisenbrey in experimental chromate nephritis. If this curve of nitrogen and chloride is super-imposed on a curve of the fluid output, it will be seen that the two follow each other closely. The nitrogen output is reduced, the chloride output is markedly reduced, yet their fluctuations correspond closely to those of the urinary secretion.

3. UREA

The urea nitrogen excretion was constantly low. The first determination made after the onset of nephritis showed a total 24 output of 6.32 gm. On December 15, the day following the marked suppression of urine, it fell to 0.81 gm. and on the following day it was only 1.2 gm. It later rose slowly, reaching the highest level on December 29 when it was 9.49 gm. During the last five days of the patient's illness it again showed a de-

cided depression, falling to 1.62 gm. on the day before the patient's death. In comparing these figures with those of the total nitrogen and non-proteid nitrogen excretion, it is seen that, while these two are markedly diminished, yet their depression is not relatively so great as that of the urea excretion.

The blood urea, on the other hand, was high at the onset—65.38 gm. on December 14, and afterwards showed a constant and gradual rise, reaching the high value of 340 mg. per 100 c. c. the day before death. For two weeks from December 2 to January 3, during the period when the patient, as previously mentioned, felt comparatively well, the blood urea varied from 168 to 227 mg.

These urea values are interesting in the light of Cushny's well-known, modern theory. Cushny states that "the modern view requires that the urea, like the other constituents of the urine, pass out by the capsule." He cites, in proof of this view, that the excretion of urea ceases at the same time as the secretion of water by the kidney. In this patient, however, the secretion of water, after an initial drop, rose rapidly and continued at a high level while the urea output remained very low and the blood urea continued to increase markedly.

Urea curves similar to that in this case have been frequently noted in cases of chronic diffuse nephritis showing at autopsy both glomerular and tubular lesions. Such urea values, as noted in our case of tubular nephritis, raise the question whether the older view, that urea is excreted mainly by the tubules, does not explain the picture better than the modern theory.

Further evidence in favor of this older view is found in the work of Oliver.¹⁴ This observer, working with xanthidrol, which produces a characteristic crystalline product with urea, found evidence of urea excretion in both the glomeruli and tubules. The marked diminution of urea excretion in our case of chromium nephritis would indicate that a part of the urea may have escaped through the intact glomeruli, while still another fraction may have been secreted by such portions of the tubular epithelium as were not destroyed.

4. CREATININ AND CREATIN

The 24 hour excretion of creatinin fell to 0.23 gm. two days after the onset of nephritis, but following this rose gradually to normal and hovered about 1 gm. for two weeks. The last few days it was again depressed, falling to 0.3 gm. the day before death.

The blood creatinin on the other hand was 4.56 gm. on December 14 and rose rapidly until the fifth day after the onset of the nephritis when it reached 14.8 mg. per 100 c. c. From this time to the end it showed a gradual rise, reaching 17 mg. the day before death. Here again the patient although comparatively free from symptoms for two weeks, showed blood creatinin values varying from 13.68 mg. to 15.68 mg. per 100 c. c., demonstrating the point emphasized particularly by Myers and Lough¹⁵ that values above 5 mg. indicate an early fatal termination and also that a progressive increase in blood creatinin is a sign of unfavorable prognostic import.

The blood creatin was increased to 12.48 mg. per 100 c. c. on the day following the partial suppression of urine and on the next day fell sharply to 5 mg. It remained about this

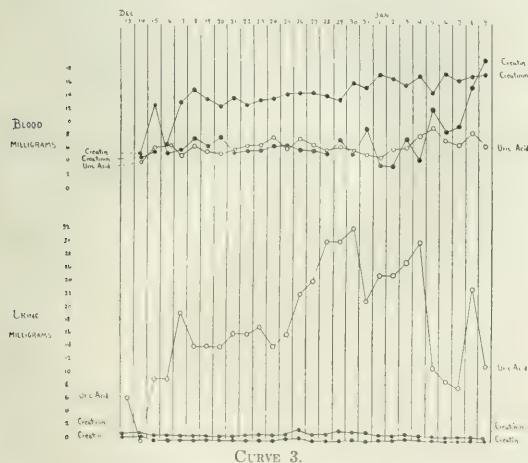


CURVE 2.

latter level until a few days before death when it rose abruptly, reaching 18.72 mg. per 100 c. c. on the day before death. The excretion of creatin varied from 0 to .17 mg.

5. URIC ACID

The blood uric acid values were constantly high, varying from 4 mg. per 100 c. c. on December 14, the second day of the nephritis, to 9.15 mg. on January 5. There was not the



CURVE 3.

same striking increase in blood uric acid as there was in the blood urea, although the amounts were increased, the average reading being 6 mg. per 100 c. c. The uric acid excretion was consistently low varying from 6 mg. to 30 mg. in 24 hours.

This patient was on a very low purine diet and the determinations show a very definite uric acid retention, which does not seem in complete harmony with the theory of Cushny which postulates a glomerular excretion of uric acid.

6. CHLORIDES

The plasma chlorides in this case of chronic acid nephritis show a consistently low level varying from 378 mg. to 558 mg. per 100 c. c. following the development of the kidney lesion. The total chlorides in the urine were even more markedly lowered, varying from .1 gm. to 2.76 gm. in 24 hours. No marked fluctuations were observed, although the general tendency was a gradual lowering in the amount excreted as the process continued. The low values were doubtless due in part to the low chloride content of the diet, and the absence of any increase in the plasma chlorides in the presence of an extensive tubular nephritis would seem to be evidence in favor of the excretion of these substances by the glomeruli.

7. SUGAR

The urine of this patient frequently showed traces of sugar which apparently bore no relation to amount of blood sugar present. The urine during the first 12 days showed traces of sugar on three occasions, although the blood sugar was not higher than 159 mg. per 100 c. c. The last four days of the patient's illness the blood sugar varied from 204 mg. to 229 mg. but with no glycosuria. Intravenous injections of glucose given on three occasions produced no marked change in the blood sugar curve.

Kossa (*l. c.*) in experimental chromate nephritis noted glycosuria in the absence of a hyperglycemia and explained this phenomenon by the assumption of an increased glomerular permeability. Cushny has attacked this theory of increased glomerular permeability used so long in phloridzin glycosuria, and has pointed out that a failure on the part of the tubular epithelium to re-absorb the excess of glucose excreted by the glomerulus explains the phenomenon equally well or better. In chronic acid nephritis with extensive tubular necrosis, such a failure of reabsorption is presumably present.

8. CARBON DIOXIDE, ACETONE, PHOSPHATES AND AMINO ACIDS

Evidence of acidosis in this patient was present soon after the onset of nephritis, the carbon dioxide tension in the plasma on December 15 falling to 37 per cent. The patient was immediately given sodium bicarbonate by mouth with the result that there was a sharp rise to 64 per cent the following day. As the result of such alkali therapy a normal carbon dioxide tension was maintained until the onset of vomiting when the tension fell to 30 per cent, and did not later rise above 37 per cent.

Acetone was present in the urine the last ten days, although it was absent in the early stages in spite of other evidence of acidosis. Determinations of inorganic phosphates in the blood were made on four occasions, showing 35 mg. per 100 c. c.

SUMMARY OF CHEMICAL STUDIES IN CHROMIC ACID NEPHRITIS

Blood milligrams per 100 c. c.																														
December															January															
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	
Non protein nitrogen.....	
Urea nitrogen.....	65	93	118	136	155	162	148	168	179	184	194	200	208	201	199	195	199	204	200	222	227	240	261	295	311	322	334	
Creatinin.....	..	4.5	5	6	13	15	13	12	14	13	13	13	14	14	14	14	16	15	17	17	16	17	15	18	17	17	17	
Creatin.....	..	5	12	5	6	6	8	5	5	5	6	6	6	6	6	5	7	5	9	3	3	8	4	12	9	9	15	19	..	
Uric acid.....	..	4	6	7	5	6	6	5	6	3.6	6	6	7	7	6	5	6	5	6	6	6	8	9	7	7	9	6	
Plasma chlorides.....	..	730	558	498	459	..	400	399	378	383	378	405	405	445	470	470	440	400	400	422	475	459	485	438	440	407	423	405	..	
Sugar.....	..	123	122	144	123	110	110	124	132	122	142	125	160	231	187	190	211	173	191	209	155	168	174	168	204	231	217	210	..	
CO ₂ in per cent.....	37	64	50	..	52	62	70	62	53	48	49	52	45	45	40	44	43	40	42	36	30	37	34	32	31	32	..	
H-ion serum ph.....	7.2	9.3	..	9.9	7.7	
Calcium.....	8.2	
Inorganic phosphates.....	33	32	30	30	
Total phosphates.....	
Amino acids.....	12.7	32	34	31	
Urine output																														
Fluid intake.....	480	280	3430	2880	3740	2770	2800	2480	3230	1140	1850	1720	1852	2232	2300	2312	2465	2570	2570	2310	1840	1640	1855	705	992	1180	550	1854	..	
Total urine.....	120	10	310	530	520	950	1100	1800	560	800	1555	1460	1460	1700	1400	1375	1800	1580	1640	1150	1150	1370	1010	560	455	370	435	280	175	
Specific gravity.....	1.025	1	1.006	1.013	1.012	1.011	1.012	1.013	1.011	1.012	1.013	1.015	1.012	1.014	1.016	1.015	1.016	1.014	1.015	1.016	1.016	1.015	1.017	1.019	1.017	1.017	1.019	1.019	..	
Albumin.....	6.9	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	..	7.3	
Total nitrogen.....	9.65	..	1.17	1.55	1.56	2.85	2.31	4.08	3.40	2.87	6.59	6.83	6.2	9.18	8.82	9.60	13.5	14.2	12.3	7.28	8.25	10.2	7.94	4.55	3.49	3.25	3.80	2.31	1.24	
Non protein nitrogen.....	7.70	..	9.6	1.45	1.46	2.8	3.3	3.55	2.5	2.5	5.3	6.29	6	9.1	8.7	9.4	13.4	14.1	12.1	..	6.8	10.1	6.98	4.15	3.29	2.21	2.65	1.8	..	
Urea nitrogen.....	6.82	..	8	1.2	8.8	2.57	2.64	3.77	1.68	2.28	2.6	5.38	3.22	4.3	3.96	6.69	9.49	7.49	8.65	6.99	6.85	8.58	6.12	9.21	2.81	2.22	2.07	1.62	73	
Ammonia nitrogen.....	31	..	120	16	31	17	04	01	01	2	55	62	70	76	70	34	74	17	017	0.67	59	82	84	67	55	52	44	36	15	
Creatinin.....	8	..	23	32	26	48	33	45	18	3	72	57	54	1.1	88	66	1.2	1.07	1.9	79	79	1.0	80	45	39	37	33	30	2	
Creatin.....	17	..	92	0	0	0	07	05	04	0	0	1	02	02	0	0	0	07	0	19	13	27	38	02	18	05	18	04	01	
Uric acid.....	6	..	0	9	19	14	14	14	16	17	14	16	22	24	30	30	32	21	25	25	27	30	11	9	8	23	12	30	..	
Total chlorides.....	2.5	..	50	79	52	114	123	136	73	80	124	1.68	1.92	2.72	2.76	1.68	1.62	1.74	1.31	81	1.11	1.10	1.76	92	14	22	26	20	71	
H-ion.....	6.8	..	5.8	2	8	8.7	7.8	8	8.2	8.2	7.9	8.5	9	8.4	8.5	8.8	8.6	9.2	9.2	9.2	9.2	9.2	8.8	9.0	8.8	8.7	8.5	7.8	..	
Sugar.....	+	
Phosphates as P ₂ O ₅	9	..	0.6	13	12	25	44	68	28	88	1.68	1.12	1.22	1.3	..	1.15	..	1.12	..	1.14	1.14	1.34	1	1.63	1.45	1.34	1.38	1	03	
Chromium.....	+	
Acetone.....	
McLean Index.....	13	12	05	69	13	14	69	68	69	..	11	17	14	3	47	35	38	34	29	37	20	06	07	04	01	01	..	

Albumin, total nitrogen, nonprotein nitrogen, urea, ammonia and total chlorides in grams.
Creatinin, creatin, uric acid and phosphates in milligrams.

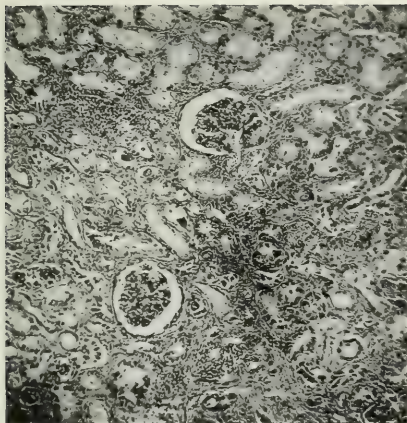


FIG. 1.—Microphotograph of the kidney showing desquamation of the tubules, interstitial fibrosis and areas of small, round cell infiltration. (Hematoxylin and eosin. Bausch and Lomb obj. 4 oc. $\times 10$.)

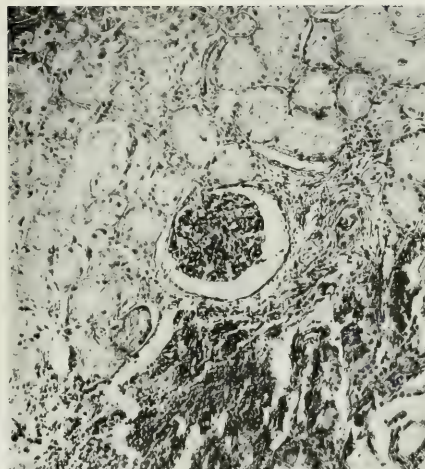


FIG. 2.—Microphotograph of the kidney showing extensive tubular desquamation with an intact glomerulus. (Hematoxylin and eosin. Bausch and Lomb obj. 4 oc. $\times 10$.)



on December 29 and 50 mg. per 100 c.c. on January 8, two days before death. The phosphate excretion in the urine was consistently low, falling to 0.1 gm. the day before death. These findings indicate an early phosphate acidosis complicated later by an acidosis of acetone body origin. Determinations of the blood amino acids were made on four occasions and showed high values, 12.7 mg. per 100 c.c. on December 30, 1920, 32 mg. on January 7, 1921, 24 mg. on January 8 and 31.3 mg. on January 9.

CONCLUSIONS

In this case of chronic acid nephritis the kidney lesion present was that of a pure tubular nephritis. No edema was noted clinically and no anasarca or ascites was present at autopsy. No symptoms of uremia were present, and the patient, during the greater part of his illness, felt comparatively well.

The urine output following a temporary depression was high, but the urine itself was of low specific gravity and the excretion of nitrogen, chlorides, phosphate, creatinin, uric acid and urea was markedly diminished. Glycosuria appeared from time to time but it bore no apparent relationship to the amounts of blood sugar present.

A study of the blood chemistry showed very high values for urea, inorganic phosphates, amino acids and creatinin and values higher than normal for uric acid. Determinations of the carbon dioxide content of the blood plasma showed definite evidence of acidosis which responded promptly to alkali therapy.

In conclusion it is a very great pleasure to acknowledge the assistance of Dr. R. L. Haden, under whose direction the chemical studies were carried out.

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ADAPTATION OF BACTERIA TO GROWTH ON HUMAN MUCOUS MEMBRANES WITH SPECIAL REFERENCE TO THE THROAT FLORA OF INFANTS

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In studying the problem of respiratory infection it soon became apparent that certain fundamental data in regard to growth and colonization of bacteria in the upper air passages must be assembled before real progress could be made. It has seemed advisable, therefore, to digress from the original practical problem to more purely biological questions of growth adaptation of bacteria on the respiratory mucous membranes.

The recent trend of bacteriological study clearly indicates that the conditions allowing and promoting the growth of microorganisms are vastly more complex than the early investigators in this domain were led to believe. The newer studies on the importance of hydrogen-ion concentration, and of growth accessory substances in particular, have opened roads which will undoubtedly lead into very extensive and prolific fields of

research. However, these considerations apply more especially to growth of bacteria on artificial nutritive media, and the facts at hand help but little in elucidating the conditions of growth of micro-organisms in the body. It seems that factors more elusive than those of acid-base equilibrium and such matters govern the actual conditions of human parasitism. To illustrate—while the pneumococcus is found in the mouths of approximately 50 per cent of normal people, we find that the pH of the mouth secretions is almost always greater than that which would allow the initiation of growth of the pneumococcus in the test-tube.¹ Furthermore, certain organisms such as *B. coli* and Friedländer's bacilli, whose growth requirements *in vitro* are simple, do not colonize on the mucous membranes of the upper air passages, whereas more fastidious organisms such as streptococci are invariably present. It is apparent, therefore, that one cannot directly apply test-tube criteria in explaining growth on human mucous membranes.

The immediate question under consideration, then, concerns the factors which allow growth of certain organisms in the upper air passages and prevent that of others. In a previous study it was pointed out that the bacteria found in the throat considered from this standpoint fall into several distinct groups.² There is in the adult (with aerobic methods) a constant habitual flora consisting of non-hemolytic streptococci, Gram-negative cocci, and diphtheroids. These organisms are found widely disseminated through the mouth cavity—on tongue, tonsils, and pharynx. They occur in normal and in sick people, in those with foci of infection, and in those whose tonsils have been removed. All the evidence indicates that they grow free on the normal mucous surfaces. In distinction to this group we find another comprising for the most part potentially pathogenic organisms. These are usually localized in some definite area of infection such as the tonsil, adenoid, or a sinus, or they may occur temporarily and sporadically on the free surfaces of the mucous membranes—evidently transients which have not actually colonized. What then are the conditions which create such a sharp and fundamental difference between these two groups of organisms? An analysis of the known facts in regard to test-tube growth requirements of the two groups does not answer this question. For example, hemolytic streptococci are no more fastidious than many of the green-producing strains, and yet the former are not members of the normal habitual flora, whereas the latter are constantly present. It seems to us necessary to fall back on a theory of adaptive parasitism, without attempting to define the exact chemical conditions involved. In other words, is it possible that the non-hemolytic streptococci gradually—perhaps during a period of thousands of years—became adapted to growth on human mucous membranes, while other bacteria show a total absence of such adaptation or only partial and abortive degrees?

It seemed that some information about this question might be gleaned from a study of the development of the throat flora in infants. Such individuals would furnish a most desirable experimental material for the following reasons: First, one would be dealing with a virgin soil not previously the habitat

of any bacteria. Secondly, no local foci of infection such as diseased tonsils or sinuses are present to obscure the bacteriological picture. Thirdly, the teeth with their associated collections of food particles in which organisms of various sorts may breed have not yet appeared. If it should turn out that any particular varieties of organisms appeared early, regularly, and in large numbers, one might reasonably assume that such organisms possessed a high degree of inherent adaptation to growth in these regions. The present paper concerns itself, therefore, with a study of the throat flora of infants.

LITERATURE

No attempt will be made to review the extensive literature on the bacteriology of the mouth. Our work concerns itself with defining the position of certain well-known organisms rather than with enumerating all bacteria which may be recovered from the upper air passages under all conditions. Brailovsky-Lounkevitch³ has reviewed the literature on the bacteriology of the mouth in infants. She, as well as other observers, has found no organisms during the first few hours of life but notes their rapid appearance after the twelfth hour. Beyond this important observation, however, we find little but a general catalogue of organisms without information which would indicate their significance.

METHODS

Through the courtesy of the staff of the Obstetrical Department it was possible to examine infants shortly after birth and at more or less regular intervals thereafter. A swab was passed over the palate and pharynx and plated on rabbit blood agar and oleate hemoglobin agar according to the method described elsewhere.⁴ Care was taken to secure a good spread of colonies and careful qualitative and quantitative observations were made of the various bacteria present. A note was made of the relation of the culture to the time of nursing.

RESULTS

The results are presented in Table I. The first fact of importance is that the cultures made very shortly after birth—under 12 hours, and before the baby had nursed—were sterile. This was so in seven cases (I, V, IX, XI, XII, XIV, XIX). In Case XVII a culture made three hours after birth and before nursing yielded four colonies of staphylococcus albus and two colonies of a diphtheroid. From the twelfth hour on (at which time nursing began) organisms were uniformly present in large numbers. In Case VII a culture made at 12 hours, but before nursing, showed a few white staphylococci and several hundred green streptococci. The above findings confirm those of previous observers.

A few words may now be said about each of the organisms encountered in later cultures.

1. *Staphylococci (albi)*.—White staphylococci were present in over half of the cultures but were not a constant finding. They varied in number in various cultures from a few to innumerable colonies. Many types, both hemolytic and non-

TABLE I
RESULTS OF THROAT CULTURES FROM INFANTS¹

Aug. 6—at, 11 hrs. (has not nursed)		Aug. 8—at, 3 days		Aug. 15—at, 10 days		Aug. 17—at, 12 days	
Case I—F....	No growth	Staph. albus 20 Tetragenus a few <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞		Staph. albus ∞ Tetragenus a few <i>Strept. (green)</i> many <i>Strept. (grey)</i> ∞ Staph. aureus 50		Staph. albus ∞ <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞	
Aug. 8—at, 14 hrs. (has nursed)		Aug. 9—at, 2 days		Aug. 15—at, 8 days		Aug. 17—at, 10 days	
Case II—E....	Tetragenus 1 Gram+ diphtheroid 1	Tetragenus many Gram-neg. cocci many <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> a few		Tetragenus ∞ <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> many Staph. albus 50		Tetragenus many <i>Strept. (green)</i> many <i>Strept. (grey)</i> ∞ Staph. albus few	
Aug. 8—at, 16 hrs. (has nursed)		Aug. 9—at, 2 days		Aug. 10—at, 10 days		Aug. 15—at, 8 days (culture while nursing)	
Case III—R....	Staph. albus 16	Spreaders		Tetragenus sev. hundred <i>Strept. (green)</i> many <i>Strept. (grey)</i> ∞		Staph. albus sev. hundred Tetragenus few <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Staph. aureus 5	
Aug. 9—at, 14 hrs. (has nursed)		Aug. 10—at, 2 days		Aug. 15—at, 7 days		Aug. 19—at, 11 days	
Case IV—Fr....	Staph. albus 200 <i>Strept. (green)</i> a few <i>Strept. (grey)</i> many	Staph. albus many <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Tetragenus ∞		<i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> a few Tetragenus ∞		Staph. albus many <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> many Tetragenus many Staph. aureus few	
Aug. 23—at, 16 hrs. (has nursed)		Aug. 24—at, 1½ days		Aug. 26—at, 4 days			
Case V—O....	Staph. albus 100 <i>Strept. (grey)</i> 50	Staph. albus 120 Gram+ coccus many Tetragenus ∞		Staph. albus few <i>Strept. (grey)</i> many <i>Strept. (green)</i> many Tetragenus many Staph. aureus a few Gram+ bacillus 6			
Aug. 1—at, 7 hrs. (has not nursed)		Aug. 2—at, 30 hours		Aug. 5—at, 3 days		Aug. 8—at, 6 days	
Case VI—S....	No growth	Staph. albus 300 Tetragenus a few <i>Strept. (green)</i> many Gram-neg. bacillus ∞		Staph. albus sev. hundred Tetragenus a few <i>Strept. (green)</i> ∞		Staph. albus sev. hundred Tetragenus a few <i>Strept. (green)</i> sev. hundred <i>Strept. (grey)</i> ∞ Staph. aureus 12	
Aug. 2—at, 12 hrs. (has not nursed)		Aug. 3—at, 31 hours		Aug. 5—at, 3 days		Aug. 8—at, 6 days	
Case VII—J....	Staph. albus few <i>Strept. (green)</i> sev. hundred	Staph. albus few <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Gram-neg. cocci few		Staph. albus ∞ <i>Strept. (grey)</i> few		Staph. albus ∞ <i>Strept. (grey)</i> few	
Aug. 3—at, 22 hrs. (has nursed)		Aug. 5—at, 3 days		Aug. 9—at, 7 days		Aug. 12—at, 10 days	
Case VIII—Sn.	Staph. albus ∞ <i>Strept. (green)</i> many <i>Strept. (grey)</i> many	<i>Strept. (green)</i> ∞ Tetragenus ∞ Gram+ diphtheroid few		<i>Strept. (green)</i> ∞ Tetragenus ∞ Gram-neg. bacillus many		Staph. albus many <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> few Tetragenus many	
July 19—at, 6 hrs. (has not nursed)		July 20—at, 31 hours		July 22—at, 3 days		July 29—at, 10 days	
Case IX—Ce....	No growth	Staph. albus 50 <i>Strept. (green)</i> ∞ Lactis aerogenes 4		<i>Strept. (green)</i> ∞ Tetragenus many		Staph. albus few <i>Strept. (green)</i> ∞ Staph. aureus few	

* ∞ = innumerable.¹ The numbers indicate number of colonies.

TABLE I—CONTINUED

	July 22 - at. 20 hrs. (has just nursed)	July 23 - at. 2 days	July 29 - at. 8 days
Case X—H...	<i>Strept. (green)</i> ∞ Tetragenus 50 Gram-neg. cocci 50 Diphtheroid few	<i>Strept. (green)</i> ∞ Tetragenus many Staph. albus 5 Staph. aureus 1	<i>Strept. (green)</i> ∞ Tetragenus few <i>Strept. (grey)</i> few Staph. albus many
	July 23 - at. 1½ hrs. (has not nursed)		Aug 2 - at. 10 days Staph. albus sev. hundred Tetragenus few <i>Strept. (grey)</i> many <i>Strept. (green)</i> few
Case XI—Hi...	No growth		
	July 14 - at. 1 hr. (has not nursed)	July 15 - at. 1 day	July 19 - at. 5 days Staph. aureus 6 Tetragenus many <i>Strept. (grey)</i> many Staph. albus ∞ Diphtheroids few
Case XII—Ch...	No growth	Staph. aureus 1 Tetragenus sev. hundred <i>Strept. (grey)</i> many	July 23 - at. 9 days <i>Strept. (grey)</i> ∞ Staph. albus ∞ Diphtheroids few <i>Strept. (green)</i> many
	Aug. 20 - at. 23 hrs. (has nursed)	Aug. 23 - at. 3 days Staph. albus 20 Gram-neg. cocci ∞ <i>Strept. (green)</i> ∞ Diphtheroids few	Aug. 28 - at. 9 days Staph. albus few <i>Strept. (green)</i> ∞ Tetragenus ∞
Case XIII—K...	Staph. albus 10 Gram-neg. cocci 20 Gram-neg. bacillvs 50 <i>Strept. (green)</i> ∞	Staph. albus 20 Gram-neg. cocci ∞ <i>Strept. (green)</i> ∞ Diphtheroids few	Staph. albus few <i>Strept. (green)</i> ∞ Tetragenus ∞
	Aug. 23 - at. 1 hr. (has not nursed)	Aug. 24 - at. 1 day	Aug. 26 - at. 3 days Gram-neg. cocci many <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Tetragenus ∞ Staph. aureus 10
Case XIV—W...	No growth	Staph. albus few Gram-neg. cocci many <i>Strept. (green)</i> many	Aug. 28 - at. 5 days Staph. albus few Gram-neg. cocci ∞ <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Tetragenus ∞
	Aug. 17 - at. 23 hrs. (culture while nursing)	Aug. 18 - at. 2 days	Aug. 20 - at. 4 days Staph. albus 200
Case XV—N...	<i>Strept. (grey)</i> ∞ <i>Strept. (green)</i> ∞ Tetragenus ∞ B. lactis aërogenes 50	<i>Strept. (grey)</i> ∞ <i>Strept. (green)</i> few Tetragenus ∞	<i>Strept. (grey)</i> many <i>Strept. (green)</i> many Tetragenus ∞ Staph. albus 200
	Aug. 14 - at. 23 hrs. (has nursed)	Aug. 22 - at. 3 days	Aug. 24 - at. 5 days Staph. albus many Tetragenus ∞ <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Staph. aureus 50
Case XVI—A.C.	Staph. albus 10 Gram-neg. cocci few Tetragenus sev. hundred <i>Strept. (grey)</i> ∞ <i>Strept. (green)</i> ∞	Staph. albus ∞ Tetragenus many <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> many Diphtheroids few	Staph. albus many Tetragenus ∞ <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞ Staph. aureus 50
	Aug. 15 - at. 3 hrs. (has not nursed)	Aug. 16 - at. 1 day	Aug. 18 - at. 3 days Staph. albus 200
Case XVII—Ca.	Staph. albus 4 Diphtheroid 2	Tetragenus ∞ <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞	Tetragenus ∞ <i>Strept. (green)</i> many <i>Strept. (grey)</i> ∞ Staph. aureus 6
	July 19 - at. 24 hrs. (has nursed)	July 22 - at. 4 days	Aug. 3 - at. 16 days Staph. albus ∞ Gram-neg. coccus ∞
Case XVIII—Fr	Tetragenus 300 <i>Strept. (green)</i> ∞ Diphtheroids ∞ Beta hemol. strept. 100	<i>Strept. (green)</i> many Diphtheroids few Beta hemol. strept. 10 Staph. albus ∞ Gram-neg. coccus ∞	Tetragenus many Staph. albus ∞ Gram-neg. coccus ∞
			Aug. 10 - at. 26 days Tetragenus sev. hund. <i>Strept. (green)</i> ∞ <i>Strept. (grey)</i> ∞

hemolytic, were encountered, the type often differing in successive cultures from the same infant. The organisms corresponded with the varieties found on the skin (see below). Their logical source is clearly the mother's skin and the milk, and this supposition is supported by the much lower incidence of white staphylococci in adults. There seemed to be no evidence of a real carrier state, but the presence of these bacteria indicates multiple transient infestations derived from nursing at four-hour intervals.

2. *Staphylococci (aurei)*.—These organisms were found in a few cultures—in no case as constant inhabitants. Their significance was clearly that of transients derived from the mother's skin and milk.

3. *Micrococcus Tetragenus*.—Organisms of this group were found in a large percentage of the cultures but by no means constantly. Two main types were encountered (1) large uniformly Gram-positive forms in clumps and groups of four, and (2) smaller forms, many of which failed to retain the Gram stain. The colony formation also varied markedly. These organisms are common skin inhabitants, which pretty clearly explains their source.

4. *Diphtheroids*.—In marked contrast with what occurs in adults, diphtheroids were found only in occasional cultures and then in small numbers. Their significance was clearly that of transients, and their ubiquity on the skin surfaces and in the mouths of adults offers a ready explanation of their source. There was, however, no tendency to extensive or permanent growth on the mucous membranes of infants.

5. *Gram-negative cocci*.—These organisms are found practically constantly and in large numbers in the mouths of adults. In sharp contrast to this observation was their incidence in infants. We found them in only four cases of the series (II, VII, X, XIII).

6. *Streptococci*.—This group was of particular interest. As is seen in the table, streptococci appeared early, usually within 24 hours, and in nearly every case were constantly present in large numbers. Two main types were encountered, first, small green-producing colonies consisting usually of elongated cocci in long chains, which exhibited many variations in form and much variability to Gram staining, and secondly, small grey colonies of intensely Gram-positive forms in short chains. No obvious source for these organisms in the infants surroundings was found save the mouths of the attendant adults.

7. *Other Organisms*.—A variety of other organisms were encountered in single instances—clearly transients of no significance—a Gram-negative bacillus (Cases VI, and VIII), *B. lactis aërogenes* (Cases IX and XV). In Case XVIII beta hemolytic streptococci were obtained in small numbers on the first two cultures. They were clearly transients and did not colonize or produce disease, but their source was obscure. Unfortunately, no examination of the mother's milk or breasts was made.

In summary, then, the infant's mouth, sterile at birth, rapidly becomes the site of profuse bacterial growth. The flora is relatively simple compared to that of adults consisting

essentially of (1) a group of organisms constantly introduced during the process of nursing and corresponding pretty clearly with that of the skin of adults—*Staphylococcus albus*, *M. tetragenus*, *Staphylococcus aureus*, etc., (2) a small group of variable transients, and (3) non-hemolytic streptococci.

With a view to confirming the source of some of the above organisms a series of cultures was made from the skin and from the breast nipples of a group of adults associated with the infants. A swab dipped in sterile salt solution was rubbed over an area about one inch in diameter and plated on the same kinds of media used for the throat cultures. The results summarized in Tables II and III indicate the source of certain of the organisms found in the infants' mouths, such as *albus*, *aureus*, *tetragenus*, etc.

TABLE II

CULTURES FROM SKIN OF ARM OF ADULTS

- M.—50 colonies *Staph. albus* (non-hemolytic).
4 colonies *Staph. albus* (hemolytic).
6 colonies *M. tetragenus*.
A few colonies of a coarse rod-like Gram-neg. bacillus.
- E.—50 colonies *Staph. albus*.
1 colony *Staph. aureus*.
Many colonies Gram-neg. coccus.
- C.—1 colony *Staph. albus* (non-hemolytic).
2 colonies *Staph. albus* (hemolytic).
2 colonies *Staph. aureus*.
Many colonies spore-bearing Gram-neg. bacillus.
Many colonies *M. tetragenus*.
- G.—Many colonies *M. tetragenus*.
A few colonies Gram-neg. cocci.
A few colonies *Staph. albus*.
2 colonies *Staph. aureus*.
- S.—∞ colonies *Staph. albus* (hemolytic).
A few colonies *Staph. albus* (non-hemolytic).
Many colonies *M. tetragenus*.
A few Gram-pos. diphtheroids.
- J.—Many colonies *Staph. albus*.
A few *M. tetragenus*.
- D.—A few colonies *Staph. albus*.
A few colonies *M. tetragenus*.
A few colonies Gram-pos. diphtheroids.
A few colonies Gram-neg. diphtheroids.
- A.—1 colony *Staph. albus*.
- E.—12 colonies *Staph. albus*.
1 colony coarse Gram-neg. bacillus.
- C.—12 colonies *Staph. albus*.
- D.—Spreaders.

TABLE III

CULTURES FROM NIPPLES OF LACTATING WOMEN

- Case 1—∞ colonies *Staphylococci* (several kinds).
Many colonies *M. tetragenus*.
Many colonies *Diphtheroids*.
A few colonies spore-bearing bacilli.
- Case 2—∞ colonies *Staphylococci* (white).
Many colonies Gram-positive spore-bearer.
Many colonies *M. tetragenus*.
A few colonies spreaders.
- Case 3—Many colonies *M. tetragenus*.
Many colonies *Staph. albus*.
Many colonies Gram-positive bacillus.

TABLE III—CONTINUED

Case 4—	OO colonies Staph. albus. Many colonies M. tetragenus. A few colonies Staph. aureus. OO colonies Gram-positive diphtheroids.
Case 5—	A few colonies Staph. albus. Many colonies Gram-negative coccus. Many colonies Yeast. Many colonies long chain—grey streptococcus.
Case 6—	OO colonies Staph. albus. Many colonies M. tetragenus. A few colonies Staph. aureus. Many colonies Diphtheroids Spreaders.

DISCUSSION

Above we have outlined the bacteriological facts elicited in the present study. A few words may now be devoted to the general questions raised at the beginning of the paper. It was hoped that further information might be obtained about the mucous membranes of the upper air passages as sites for bacterial growth and the nature of their relation to the organisms found. It may be recalled that in healthy adults we deal with an apparently habitual constant and completely adapted flora, with transients, and with foreign organisms associated with acute disease or chronic foci of infection. The question of another group of partly adapted organisms has been raised in another place⁹ and evidence of their occurrence collected. In the present work it is of interest to note the absence of such organisms as influenza bacilli, pneumococci, and hemolytic

streptococci which are relatively frequent in adults. Without direct contact observations no definite conclusions can be drawn, but the absence of these organisms raises the possibility of an inherent lack of complete adaptation to free growth in the throat, and suggests that special conditions are necessary for their colonization.

Of major importance, however, from the present point of view is the practically constant finding of non-hemolytic streptococci in large numbers beginning within a few hours after birth. One seems forced to conclude that an inherent adaptation to free growth on the mucous membranes of the upper air passages exists in the case of this group of bacteria. We state the matter in this way at the present time, for as yet no exact information is available as to actual chemical or biological factors which allow the colonization of this group rather than that of hemolytic streptococci or a host of other organisms. These observations do, however, seem to give a hint that subtle biological adaptations are of importance in explaining the presence of bacteria under certain conditions, rather than the cruder and more specific chemical facts of bacterial growth demonstrable in the test-tube.

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DERMOID CYSTS OF THE OVARY

A REPORT OF FOUR CASES

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1. A small dermoid cyst accidentally discovered by needling an enlarged ovary.

2. A large dermoid of the left ovary; a small dermoid cyst of the right ovary.

3. A dermoid cyst in the wall of a large multilocular ovarian cystadenoma.

4. A spinal-cell carcinoma developing in a dermoid cyst of the ovary.

These four cases of dermoid cyst of the ovary are presented not because such growths are rare, but because the pathological specimens came to our laboratory in rapid sequence and each case presented something of interest either clinically or pathologically. The case histories and pathological findings in brief are as follows:

CASE 1.—G. G., a white female, unmarried, 24 years old, was operated on by Dr. Cullen at the Church Home and Infirmary, November 15, 1920. The patient had had several attacks of pain localized in the right iliac and the inferior portion of the right lumbar quadrants. Some of these attacks had been associated with nausea, vomiting and a chill. The history was otherwise irrelevant. On account of the

iliac pain the patient herself asked for a careful examination of the ovary.

The physical examination was essentially negative except for definite abdominal tenderness and muscle spasm on deep palpation over McBurney's point. A pelvic examination was not made.

At operation the appendix was found to be involved in a well-marked chronic inflammatory process. It was removed. The right tube and ovary were next brought to view through the (gridiron) incision. The tube appeared to be normal. The ovary, however, was about half as large again as normal, and presented several large follicular cysts. Three of these were punctured with a straight intestinal needle, and all yielded a clear, colorless fluid. The ovary, however, being still somewhat too large, the needle was passed deeply into its median half where no definite cyst was apparent, and immediately there escaped a very small drop of an oily fluid, which against the white surface of the ovary and on account of the good illumination appeared as a small, shimmering globule. The diagnosis being evident, an elliptical incision was made over the surface of the ovary and a dermoid cyst about 3 cm. in diameter was at once encountered. This was shelled out intact, about two-thirds of the ovary being left. The left ovary was normal.

Pathological Examination (Gyn. Path. No. 26372).—The specimen consists of a small tumor mass measuring 3.5 x 3 x 3 cm. (Fig. 1).

About one-half of this mass (marked "dermoid cyst.") has a regular, pale gray surface with a few fine shreds of pale gray tissue attached. On palpation this portion is of semi-fluid consistence and possibly is slightly fluctuant. The other part, which represents the portion of the ovary included in the elliptical incision, appears elevated, pale red, smooth, glistening and translucent. On section a thin-walled cyst is seen filled with a gray, granular, greasy material which is soft and contains a few strands of hair. In one portion of the wall is an elevated, thickened area from which a tuft of hair projects. The cyst wall is as thin as tissue paper except over the portion where some ovarian tissue is attached; here it is 3 mm. thick and has several smooth-walled, cystic areas in its substance.

Microscopic examination shows the picture of an ordinary simple dermoid cyst lined with stratified epithelium several layers thick. In the wall of the cyst are numerous hair follicles and sebaceous glands. In its cavity are pink-staining detritus and some strands of hair. The cystic areas to one side are thin-walled structures lined with a single layer of flat or low cuboidal epithelium. These are enlarged Graafian follicles.

Comment.—The point of particular interest in this case is the discovery of a dermoid cyst embedded in the substance of an ovary so as to be entirely surrounded by ovarian tissue. Careful scrutiny of the fluid obtained by paracentesis led to the discovery of a dermoid cyst which would otherwise have been overlooked, and the patient would very probably, at some later date, have been compelled to submit to another abdominal operation. The value of paracentesis ovarii as a diagnostic procedure is well brought out in this case and can equally well be applied to other selected cases of small ovarian enlargement when it is desirable to be particularly conservative in preserving ovarian tissue and when the spilling of some of the cyst contents will not lead to peritoneal implantations as sometimes happens in ovarian papillary cystadenomata.

CASE 2.—M. M. (Gyn. No. 26756), a white, married woman, 2-para, 39 years old, was admitted to The Johns Hopkins Hospital April 6, 1921, complaining of pain in the lower part of the abdomen. There was nothing of importance in the family or personal history. The menstrual history was normal.

Present Illness.—For about a year the patient had complained of a dull pain in the left iliac quadrant and gradually had become conscious of a mass in this situation which seemed to be enlarging, so that finally it gave rise to a sense of fullness whenever she sat down.

The physical examination was essentially negative except for the presence of a firm, rounded, smooth mass which was movable and apparently about 15 cm. in diameter. This mass was situated in the left iliac fossa and extended upward to a point mid-way between the umbilicus and the symphysis pubis in a position in front of and to the side of the uterus, which appeared to be essentially normal. The right ovary was in its usual position and seemed to be about twice the normal size.

At operation, April 9, 1921, the left ovary was represented by a large tumor mass lying in the left iliac fossa and twisted on its pedicle through an arc of 90° (Fig. 2). The left tube and ovary were removed in the usual manner. The right ovary also contained a yellowish cyst, through the wall of which some hair could be seen. This cyst was resected, leaving *in situ* about what would correspond to one-third of a normal ovary. The usual prophylactic appendectomy was performed and the uterus was left in good position by a modified Coffey suspension.

Pathological Examination (Gyn. Path. No. 26756).—The resected portion of the right ovary measures 4.5 x 3 x 2 cm. It is smooth, pale gray and glistening except for one area where the surface has some small vesicle-like areas which are yellow, glistening and translucent. The tumor is doughy in consistence. On section (Fig. 2; insert) a

cystic structure is seen filled with a sebaceous material in which there is some hair. The hair grows in a tuft from the inner aspect of the roughened yellow area noted on the external surface. The lateral portion of the mass, where it has come in contact with the normal ovarian substance, has several small, yellow, cystic areas.

The tumor mass on the left measures 12 x 10 x 9 cm. and has attached to it a normal Fallopian tube. The tumor is smooth, glistening, reddish, and a fine tracery of blood vessels is seen over its surface. It is doughy in consistence and on section presents the appearance of an ordinary dermoid cyst with very little hair.

Sections of the tumor removed from the right ovary show a thin wall, the inner aspect of which is lined with a stratified squamous epithelium, without papillae, beneath which are numerous hair follicles. The yellow cystic areas seen in the gross are small cavities lined with stratified epithelium and here one also sees numerous multinuclear giant cells scattered in the stroma forming the cyst wall.*

Sections from the large cyst on the left show a similar inner epidermal lining, without papillae but with an occasional hair follicle and sebaceous gland. Numerous epidermal inclusions are seen, some of these having the appearance of undifferentiated sweat glands.

Comment.—This patient passed through an uncomplicated convalescence and when last heard of had been menstruating normally at 26-day intervals. The case affords a good illustration of the feasibility of conserving the ovarian function when some normal tissue is present, thereby avoiding the danger and inconvenience of a premature operative menopause. The dermoid cysts are interesting in that they replaced about five-sixths of what should have been normal ovarian stroma; nevertheless, the patient has had and is still having her normal catamenia.

CASE 3.—L. T., a white woman, 35 years old, married, 3-para, was admitted to the Church Home and Infirmary March 27, 1921, complaining of an abdominal tumor and indigestion. The family and personal history have no bearing upon the case; the menstrual history had been normal.

Present Illness.—One year before admission the patient first noticed that she had an abdominal tumor which had gradually become larger.

The physical examination was essentially negative except for the abdominal findings. A large, symmetrical, rounded, and freely movable tumor mass was felt in the hypogastrium extending from the pelvis up to the umbilicus. This tumor could not be differentiated from the uterus on pelvic examination and we thought we were dealing with a freely movable myomatous uterus.

Operation.—The patient was operated on by Dr. Cullen on March 28, 1921. A small quantity of free straw-colored fluid was found in the pelvis. The right ovary was represented by a large ovarian cyst, which with its Fallopian tube was removed. Convalescence was uneventful and the patient is now well and free of all symptoms.

Pathological Examination (Gyn. Path. No. 26733).—The specimen consists of a large blue-domed mass to which is attached a Fallopian tube. The latter measures 10 x 1 cm. It is flattened out and stretched over the tumor mass. Its surface is covered by a smooth and glistening serosa, its lumen is patent and its fimbriae are delicate. A small appendix vesiculosa is present.

The tumor mass measures 20 x 17 x 10 cm. Its surface has a bluish sheen, is smooth, glistening, and slightly irregular in outline. Palpation elicits definite fluctuation. On section a large multilocular cystic structure (Fig. 3) is seen. It was filled with a thick, tenacious,

* Dr. Cullen has frequently drawn attention to these giant cells and he feels that they are the precursors of the squamous epithelium. He goes further and states that "wherever an alveolar structure is noted in an ovary and where these alveolar spaces are more or less lined with giant cells, one can be absolutely certain that a dermoid cyst exists in that ovary."

glistening, glairy, translucent, mucinous material. One area in the cyst opposite the tube has a rather honey-combed, bubble-like appearance and here the contents are particularly viscous. On the inferior portion of the posterior wall of the cyst is seen an encapsulated mass different from the remainder of the cyst and measuring on its cut surface 1.7 x 1.5 cm. (Fig. 3; insert). It is easily recognized as a dermoid cyst containing some yellowish-gray, greasy material and a few strands of hair which do not appear to grow as a tuft from any one situation.

Sections from various portions of the cyst wall show a scanty stroma with numerous capillaries and occasional areas of round and polymorphonuclear cell infiltration. The inner aspect of the cyst wall is lined with a single layer of columnar epithelium which only in a few places is thrown up into a low fold. The individual cells are goblet-shaped and have a pink-staining cytoplasm with a pale central zone and a well-defined cell outline. The nuclei are at the base of the cells, are oval or round and in some cells are crescentic in shape. The cells rest on a poorly defined membrana propria. The smaller cystic areas mentioned in the gross description contain a moderate quantity of a homogenous, pink-staining material in which are large vacuoles. In short, it is a picture of a typical multilocular cystadenoma. Sections of the dermoid cyst taken through its wall where it borders the cavity of the multilocular ovarian cyst (Fig. 4) show the dermoid lined with several layers of cuboidal epithelium. In its wall are hair follicles as well as sweat and sebaceous glands. In the cavity of the dermoid is some detritus in which can be identified some strands of hair. The dermoid is separated from the cavity of the adjoining cyst by a narrow strand of pink-staining fibrous stroma which in turn is lined with a single layer of columnar epithelium similar to and continuous with the previously described epithelium lining the remainder of the multilocular cyst. No recognizable ovarian stroma is seen.

Comment.—This is the first time in the history of this hospital, as far as the records of the gynecological and pathological departments show, that a dermoid cyst has been seen in association with, and included in, the wall of a multilocular pseudomucinous cystadenoma of the ovary. Although pseudomucinous cysts of the ovary are not particularly rare and dermoid cysts of the ovary are even less uncommon. Nevertheless, this particular pathological association is very unusual and the literature on it is quite meagre.

CASE 4.—E. R., a white woman, married, 37 years old, 3-para, was admitted to the Church Home and Infirmary complaining of a "lump in her stomach" and was operated on by Dr. Cullen, March 1, 1921. There is nothing of importance in the family or personal history. Her menstrual history is apparently normal.

Symptoms of the present illness were first noted in December, 1920, when she began to have discomfort in the hypogastrium, and a sense of pressure, most noticeable near the rectum, which was aggravated by exercise and by the sitting posture. Lying down gave marked relief. The patient had felt a tumor mass in her abdomen about two months before entering the hospital and she was certain that the tumor had definitely, though slowly, increased in size.

The physical examination was essentially negative except for the abdominal findings. The liver edge was felt just below the costal margin, being sharp, of about normal consistence and not tender. The lower half of the abdomen was quite tender, especially in the left iliac quadrant. Here a firm, tender, mass was felt extending from the left iliac fossa upwards to within 3 cm. of the level of the umbilicus. This same mass could be felt behind the cervix and was only moderately movable on bi-manual manipulation.

At operation a quantity of oily fluid containing white flakes and grumous-like material was found free in the peritoneal cavity. The site of the left ovary was occupied by a ruptured cyst which extended out into the left side of the pelvis and lay upon the rectum behind, its anterior and medial surfaces being adherent to the posterior

aspect of the uterus. This tumor mass with its attached Fallopian tube was removed as completely as possible, but a portion was left attached to the left side of the cervix. The peritoneal cavity was drained with two pelvic and two abdominal cigarette drains.

The patient had an intermittent pyrexia up to 101° F. for two days after the operation. At times she was jaundiced, bile was found in her urine, and she had constant rectal pain up to the time of her discharge from the hospital, March 27, 1921. She failed rapidly and died at her home June 21, 1921.

Pathological Examination (Gyn. Path. No. 26643).—The specimen consists of a Fallopian tube attached to a tumor mass (Fig. 5). The Fallopian tube, 7 x 0.5 cm., is pale gray, smooth, and glistening; its fimbriae are delicate. The tumor mass measures 10 x 8 x 7 cm. It occupies the position of an ovary in relation to the tube to which it is attached by a meso-ovarium which has a normal appearance. Its outline is rather irregular and it has several distinct nodular areas. The portion of the tumor adjoining the proximal part of the tube is pale gray, smooth, glistening, and fluctuant, while the part inferior to the distal segment of the tube is pale gray, and firm, with opaque yellow nodular areas. The most dependent of these nodular areas, *a*, has a roughened surface which shows some loss of substance, just at the point where the tumor had been adherent to and blended with the cervix. On section the tumor is seen to be a unilocular cyst with a dirty brown liquid content in which are seen small, flattened yellow masses and large quantities of hair. Its walls measure from 1.5 mm. to 3.5 cm. in thickness; they are smooth and somewhat irregular, the irregularities corresponding to the nodular areas seen externally. At its thickest part, *a*, the external surface is rough and shows some loss of substance, while its internal aspect has several tufts of hair arising from it. The cut surfaces of this area are yellow, opaque, and granular, with pale gray, glistening and translucent columnar areas, which appear to be invaginations or ingrowths from the external surface. The other areas of nodular thickening show quite smooth surfaces both externally and on section.

In sections taken through various portions of *a* the cyst is lined with a stratified epithelium, eight to ten cells thick. These cells are round and oval, their nuclei take a fairly deep blue color with the hematoxylin and eosin stain and most of them have well-defined nucleoli. Each nucleus is surrounded by a small quantity of eosin-staining cytoplasm. The cells are irregular in size, shape, and staining reaction, large mononuclear forms and mitotic figures being common. There is no papillary arrangement of the epithelium, no stratum germinativum as such is observed, nor are any hair follicles, sebaceous or sweat glands seen. As one follows the epithelium along from the thinner portion over into the thicker part of the cyst wall, a marked metaplasia is noted and the cells invade the cyst wall in long strands and solid alveoli (Fig. 6). Much central necrosis is present in the alveoli and many bizarre degeneration forms are seen. Numerous epithelial pearls, giant cells and mitotic figures also occur. The tumor in places occupies the entire cyst wall and penetrates it at one point over a fairly broad area. The predominant type of tumor cell is round or polyhedral, with a nucleus which has a finely granular chromatin distribution with a well-defined, dark-blue-staining nucleolus and is surrounded by a moderate quantity of cytoplasm. This cytoplasm takes a faint eosin stain or no stain at all and is surrounded by a well-defined pink cell membrane.

Sections through *b* (Fig. 5) show a picture similar to that seen in the sections taken from *a*, with the exception that no epithelial pearls are observed and the neoplastic process, while involving the entire thickness of *b*, does not penetrate the serosa. The epithelium lining the inner surface of the cyst about the periphery of this nodule (Fig. 7) is stratified, three to four cells thick; it shows no evidence of malignant metaplasia, being identically the same as the epithelium from other non-malignant portions of the cyst wall.

Sections through *a* (Fig. 5) show a picture similar to those from *b*, there being no microscopic continuity between the two neoplastic processes.

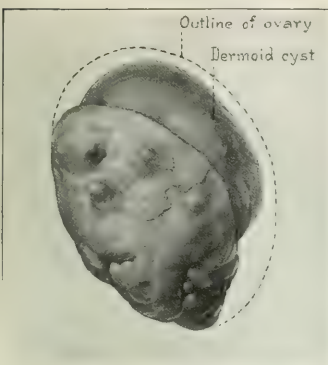


FIG. 1.—Gyn. Path. No. 26372.

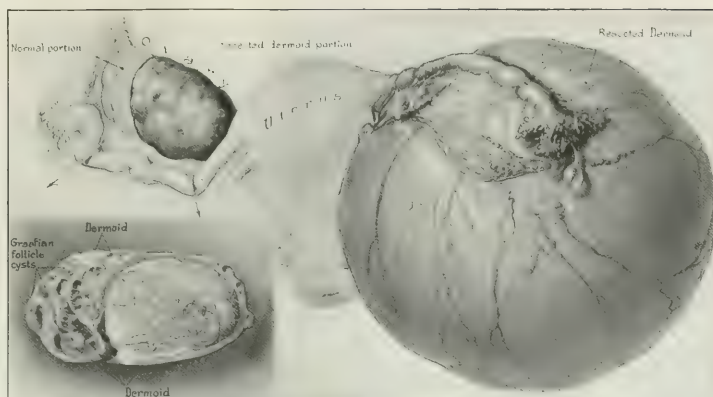


FIG. 2.—Gyn. Path. No. 26756. Case 2, showing the dermoid with its twisted pedicle on the left and the small quantity of normal ovarian tissue left on the right side after the resection.

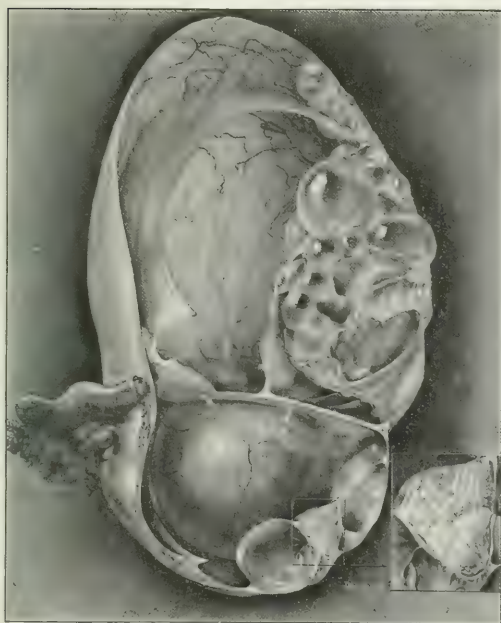


FIG. 3.—Gyn. Path. No. 26733. Case 3, a multilocular pseudo-mucinous cystadenoma of the ovary containing a dermoid cyst.

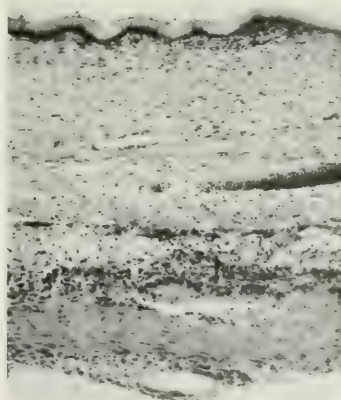


FIG. 4.—Case 3 (Gyn. Path. No. 26733). From the dermoid cyst where it adjoins the cystadenoma. The columnar epithelium lining the cystadenoma is seen at the superior margin of the picture and the wall of the adjacent dermoid at the inferior margin.



FIG. 5.—Gyn. Path. No. 26643. Case 4. Spinal cell carcinoma in a dermoid cyst of the ovary.

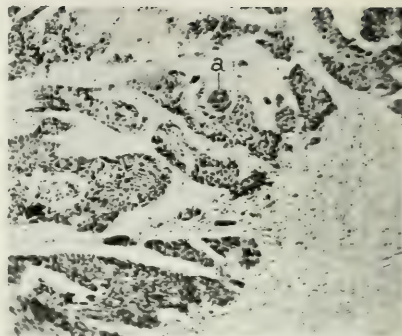


FIG. 6.—Case 4 (Gyn. Path. No. 26643). Section from Fig. 5 (a) showing spinal cell carcinoma with epithelial pearl at (a).

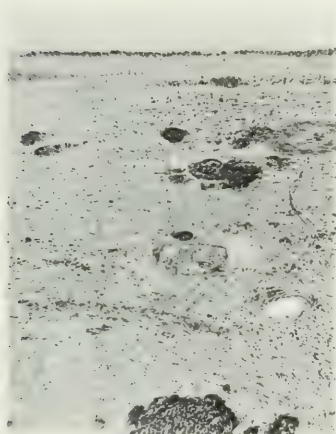


FIG. 7.—Case 4 (Gyn. Path. No. 26643). Section from nodule (b). (Fig. 5.) At the upper margin is the epidermal lining of the cyst, being here only a few cells thick and showing no evidence of malignant change. Two low power fields to the right of this area the epithelium assumes the character of that seen in Fig. 8 and invades. At the most inferior part of the photomicrograph is seen a small clump of cancer cells. This picture is taken from the periphery of (b) to show the benign appearance of the epithelium which completely surrounds these carcinomatous nodules.

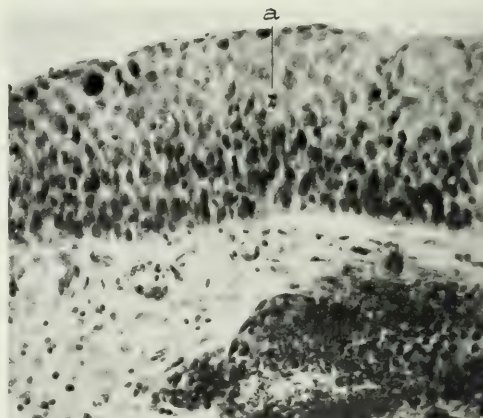


FIG. 8.—Case 4 (Gyn. Path. No. 26643). From the cyst wall adjacent to the meso-ovarium showing the thickness of the epithelium without invasion. These cells under high power show numerous mitotic figures and marked irregularity in size, shape, and staining reaction. Mitotic figure at (a).

The sections of the Fallopian tube and the meso-ovarium present nothing noteworthy. However, the adjacent wall of the cyst is lined with stratified epithelium, eight to twenty-five cells thick, which do not invade but are irregular in size, shape and staining reaction and show frequent mitotic figures (Fig. 8).

Comment.—It is interesting to note that while sections through *a* (Fig. 5) show many epithelial pearls, they are not seen in any of the others. The latter, however, show more well-defined cells and much less evidence of degeneration than at *a*. The carcinomatous change in various isolated portions of the cyst with intervening areas of non-malignant epidermal cells is striking and raises the question whether these small malignant nodules represent lymphatic metastases or whether they indicate separate foci of independent malignant change. The latter does not seem at all improbable when one considers the apparent malignant metaplasia without invasion noted in the epidermal cells (Fig. 8) lining the cyst in the portion adjacent to the meso-ovarium.

A simple dermoid cyst of the ovary, without evidence of a more complex teratomatous structure, undergoing malignant change, is in our experience very rare. Out of a total of some

200 patients who have had dermoid cysts in one or both ovaries (confirmed by examination of microsections) we have had only one other authentic case of carcinoma developing in an ovarian dermoid cyst. This occurred in 1899 in a white woman, 46 years old, who was lost track of. She was operated on, her convalescence proved unsatisfactory and she was not expected to live more than a few months after she left the hospital. It is reasonable to assume that she died as the result of the carcinoma. Sections showed a well-defined spinal cell carcinoma occurring in a dermoid cyst. The incidence of carcinomatous change occurring in a dermoid cyst of the ovary in this clinic is one per cent and our mortality from this disease is, we feel safe in saying, one hundred per cent.

I wish to express my indebtedness to Mr. Max Brödel and his pupil, Miss A. K. Lovett, for their excellent illustrations.

ADDENDUM

The value of puncture of the ovary as a practical diagnostic procedure was again demonstrated by Dr. Cullen in January, 1922, in the case of a young woman, C. H. I., No. 27512, whose history, operative findings, and treatment are almost a replica of Case 1 in this article.

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A STUDY OF THE RELATION OF THE ADRENAL GLANDS TO EXPERIMENTALLY PRODUCED HYPOTENSION (SHOCK); WITH A NOTE ON THE PROTECTIVE EFFECT OF PRELIMINARY ANESTHESIA

By ARNOLD RICE RICH

(From the Department of Pathology, The Johns Hopkins Medical School)

In a study of the literature dealing with either surgical shock or the adrenal glands, one meets frequently the suggestion that shock may be the result of disordered function of the adrenals. This idea has been entertained especially as a corollary of the belief that, since epinephrin injected intravenously has such a remarkable effect upon the blood pressure, and removal of the glands brings about a condition of hypotension, the adrenals are therefore probably concerned in the maintenance of the blood pressure at the normal level. Since the condition of shock is characterized most strikingly by a marked fall in blood pressure, it has been suggested repeatedly that epinephrin exhaustion or adrenal fatigue may be causative factors.

Stewart and Rogoff¹ and others^{2,3} have shown that stimulation of the splanchnic nerves brings about an increased output of epinephrin from the adrenals. Hoskins and McClure⁴ state that a similar effect can be produced by visceral exposure and operative trauma; Cannon and Hoskins⁵ found that

sensory stimulation increases the output of epinephrin, and Elliott⁶ showed that the glands can in this way be quite depleted of their epinephrin content. Thus, it might be supposed that excessive intestinal or peritoneal manipulation or any severe prolonged sensory stimulation accompanying trauma may exhaust the epinephrin content of the adrenals and so bring about the condition of low blood pressure characteristic of shock. Indeed, the experiments of Corbett⁷ and of Cannon and Hoskins⁸ led them to make just this suggestion, although Cannon later⁹ stated his belief that during shock "the adrenal glands are, if anything, over-active rather than exhausted." Quite a number of workers have reported that the epinephrin content of the adrenals is diminished by prolonged anesthesia, and it has been suggested that surgical shock may be dependent in part upon this epinephrin depletion.¹⁰

Numerous attempts have been made to determine whether the supply of epinephrin is actually exhausted during shock, and whether abnormal variations in the epinephrin output

from the adrenals can be related to the development of shock. The results of these investigations are somewhat conflicting. Bainbridge and Parkinson¹ reported that they could find no epinephrin at all in the adrenals taken from fatal cases of post-operative shock, and this was confirmed by Elliott.² Short,³ however, using a very delicate test, was unable to detect any reduction in the epinephrin content of adrenals taken from shock cases. Corbett⁴ states that "the symptoms of shock fully develop only after the supply of epinephrin is greatly depleted," but he does not describe the experiments which led him to this conclusion. He does, however, report that animals which were subjected to prolonged sensory stimulation (sciatic) "very rapidly went into shock after a few minutes of peritoneal trauma" when the intestines were subsequently exposed, and he explains this by assuming that the preliminary sensory stimulation caused a reduction of the epinephrin supply. It is noteworthy in this connection that, in spite of numerous attempts, there is not a single convincing experiment on record in which true shock has been produced by the stimulation of sensory nerves. Sydenstricker, Delatour and Whipple⁵ state that in animals which had been brought into the condition of shock by the injection of contents from a closed duodenal loop, determination of the epinephrin content of the adrenals disclosed only one-fourth of the normal amount, or even less. Bedford⁶ reported that during shock produced by intestinal manipulation the epinephrin content of blood taken from the adrenal vein is much higher than before the onset of shock, and that the concentration increases with the prolongation of the low blood pressure. Stewart and Rogoff,⁷ however, repeated Bedford's experiments with modifications in technique which they believe insure a greater accuracy in the estimation of the amount of epinephrin present in the blood, and they conclude that the output of epinephrin from the adrenals is the same during shock as it is under normal conditions.

Experiments such as these leave the question of the relation of the adrenals to shock in an unsatisfactory condition. In the first place, an altered epinephrin content of the adrenals or an abnormal output during shock might easily be a result rather than a cause of the condition. Thus Mann,⁸ stating his belief that the adrenals enter as factors in the complex of shock, wrote that "it is quite difficult to determine to what degree they participate as primary agents in producing the state or how much they are affected by the low blood pressure and the changes incident to the condition itself." It might be thought that the removal of both adrenals would throw immediate light upon the theory of adrenal exhaustion; and indeed Crowe and Wislocki⁹ have reported that following complete removal of both adrenals the animal gradually develops hypotension, rapid pulse, lowered body temperature, muscular weakness, apathy and dulled sensibility—all of which are characteristic of shock. On the other hand, it is claimed that adrenal extirpation does not really reproduce the condition of shock,¹⁰ and Vincent¹¹ has even stated that experiments carried out in his laboratory demonstrate that a fall of blood pressure is not a characteristic effect of complete adrenalectomy. Furthermore,

apart from the question of the effect of the mere removal of the adrenal glands, it is well known that there is a strong tendency at the present time to believe that the adrenals normally play no direct part in regulating vascular tone,^{12, 13, 14, 15, 16} or else that their activity is of value only in emergencies when an unusual strain is thrown upon the circulation. If the latter supposition be true, it is clear that exhaustion of the adrenals during the strain of an operation or severe trauma might deprive the animal of a protection of which the adrenalectomized animal, lying quietly in its corner, would have no need. Indeed, Abelous and Langlois¹⁷ pointed out long ago that muscular exertion following adrenalectomy hastens the development of the characteristic symptoms of adrenal deficiency. Thus it is evident that conclusions drawn from the effects of adrenalectomy alone cannot entirely satisfy the question of the relation of the adrenal glands to shock. The present experiments were undertaken in the belief that if normal animals, subjected to a standardized trauma, fall into shock in a reasonably definite period of time, then worthwhile information might be obtained from a comparison of the protocols of these normal controls with the time required for the production of shock in adrenalectomized animals, subjected to the same trauma. For if shock results from epinephrin exhaustion or adrenal fatigue, the condition would presumably develop much more rapidly in the adrenalectomized animals than in the normal controls; if abnormal stimuli going to the glands cause an excessive output of some harmful material which produces shock, removal of the glands would remove the possibility of an outpouring of any noxious secretion and the adrenalectomized animals would be very resistant to the trauma; and finally, if the development of shock is independent of the activity of the glands, the adrenalectomized animals should react as normal animals to the same trauma. The fact that bits of accessory adrenal and chromaffin tissue are present in animals has not been overlooked, but it was believed that the removal of both adrenal glands (the great bulk of such tissue) would produce a deficiency sufficient to be detected by this method of attack if adrenal function actually plays an important rôle in the production of shock.

EXPERIMENTS

Certainly one of the most confusing things that one meets throughout the literature concerned with shock is the failure of many workers to standardize their experiments. Often, in a series of experiments where such precautions would be of value, no attempt is made to treat each animal in as nearly the same manner as possible either as regards the degree of trauma inflicted or (what is exceedingly important) the amount of anesthetic administered during the experiments. But especially is there a lack of a criterion as to what shall be regarded as shock. Thus, while most clinical and experimental observers incorporate a marked fall of blood pressure into their definition of shock, there are some whose experiments indicate that they do not regard such a fall of blood pressure as a necessary part of the picture. Wiggers¹⁸ considers a fall of blood pressure a characteristic of "shock" produced by intestinal exposure,

but he does not consider it an essential part of the "central nervous system shock" which he produced by sensory stimulation.¹⁸ This "central nervous system shock" is merely a condition of apathy and dulled sensibility and can occur with no important blood pressure fall. Doubtless some of the confusing experiments in the literature have arisen from a failure to recognize that this condition of apathy is not true shock. Mann¹⁹ some years ago called attention to the need of a criterion for the condition of an experimental animal which should be regarded as shock, and his requirements followed admirably the signs which make up the clinical condition designated as shock.

In the present experiments, an animal was considered to be in shock when the blood pressure, having fallen to 60 mm. of mercury or below, showed no tendency to recovery and was accompanied by a permanent dulling of the sensibility, so that the ether could be discontinued without discomfort to the animal during operative procedures performed after a lapse of time (half an hour) sufficient to allow the anesthetic effect of the ether to wear off. Strong sensory stimulation applied to such an animal might cause struggling, but the animal would at once sink back into its quiet, apathetic state as soon as the stimulation was discontinued, although the abdominal contents were exposed and the neck opened for tracheotomy and cannulation of the carotid artery. A low blood pressure was required in these experiments because clinical shock without hypotension, although spoken of by a few writers,²⁰ is certainly an anomalous condition except in cases following head-wounds, and it is questionable whether such conditions are fundamentally the same as ordinary shock with hypotension. The level of 60 millimeters of mercury was arbitrarily chosen as a standard after a study of the average unrecoverable fall of blood pressure usually accompanied by a permanent dulling of the sensibility. The pulse of the animal in shock was usually rapid and feeble after the condition had persisted for some time, but frequently a marked slowing of the pulse was a prominent feature of the early stages, and periods of bradycardia have been observed during deep shock. The character of the respirations was very variable in the different experiments. The superficial reflexes remained active throughout, and the body temperature always fell several degrees centigrade more than did that of normal animals merely kept under ether anesthesia for the same period of time.

In all of the experiments described below apparently healthy, full grown cats were used and a careful attempt was made to subject each animal to conditions as nearly identical as possible as regards the trauma inflicted to produce shock and the amount of anesthetic used in each experiment. It must be stated here that during the progress of this work a number of animals were encountered which exhibited a peculiar sensitiveness to ether, so that the most careful administration of the anesthetic did not prevent them from behaving in a most anomalous way. The respirations would cease, and the blood pressure would fall abruptly shortly after anesthetization, and although artificial respiration would soon restore them, such lapses would occur repeatedly, and shock would appear much

sooner than in normal animals. At autopsy no reason for the peculiar reaction was ever discovered. The number of such animals was relatively small, and it is felt justifiable to exclude them entirely from consideration, since they were clearly hypersensitive to the effect of ether alone.

Reaction of Normal Animals to Intestinal Manipulation.—The first series of experiments was carried out to determine whether normal animals would exhibit any constancy in the time required for the development of shock if they were subjected to a uniform trauma. Each animal was anesthetized with ether and tracheotomized. The tracheal cannula was connected through a reservoir with a tube through which ether vapor could be blown from an ether bottle by means of a foot bellows. As soon as the carotid artery could be cannulated and connected with a mercury manometer, a blood pressure tracing was made and the rectal temperature was recorded. Then the abdomen was opened at once along the midline, and the small intestines were lifted out of the abdominal cavity and spread upon gauze pads, care being taken to prevent torsion of the mesentery from interfering with the circulation. Every five minutes a blood pressure tracing was made, the temperature was recorded, the intestines were pinched firmly between the thumb and forefinger all the way from the duodenum to the cæcum once, and the animal received the amount of ether vapor which was forced into the reservoir by a single pump of the bellows. In the intervals between these procedures the animal was left to lie quietly. Usually a single pump of ether vapor sufficed to keep the animals anesthetized, and a second dose between the five-minute periods was administered only when necessary. The anesthesia was never deepened enough to abolish the corneal reflex. A careful control of the anesthetic is of great importance in such experiments, as may be seen from a consideration of Fig. 1. In this experiment a relatively large amount of ether was administered, with the result that the blood pressure fell to the shock level within a short while, and would have remained there, simulating the condition of shock, had not the ether been discontinued. Fifteen minutes after stopping the anesthetic the blood pressure began to rise and 10 minutes later had risen 40 millimeters more and the animal had regained full consciousness and sensibility. This effect of ether was repeated several times in the same animal, and demonstrated the necessity, in every experiment, of observing the animal for at least half an hour following the discontinuance of the anesthetic when shock is believed to have developed, in order to be certain that the low blood pressure and dulled sensibility are not ether-effects.

By following carefully the method outlined above, each animal received as nearly as possible the same degree of trauma and the same amount of anesthetic.

Inspection of the protocols and blood pressure tracings of this series of experiments shows that normal animals react to these conditions in a strikingly constant way. The blood pressure falls abruptly during the first five minutes after exposing the intestines and then, either continuously or after a temporary rise, it sinks gradually, with some irregularity, to the shock level, and the characteristic signs of shock make

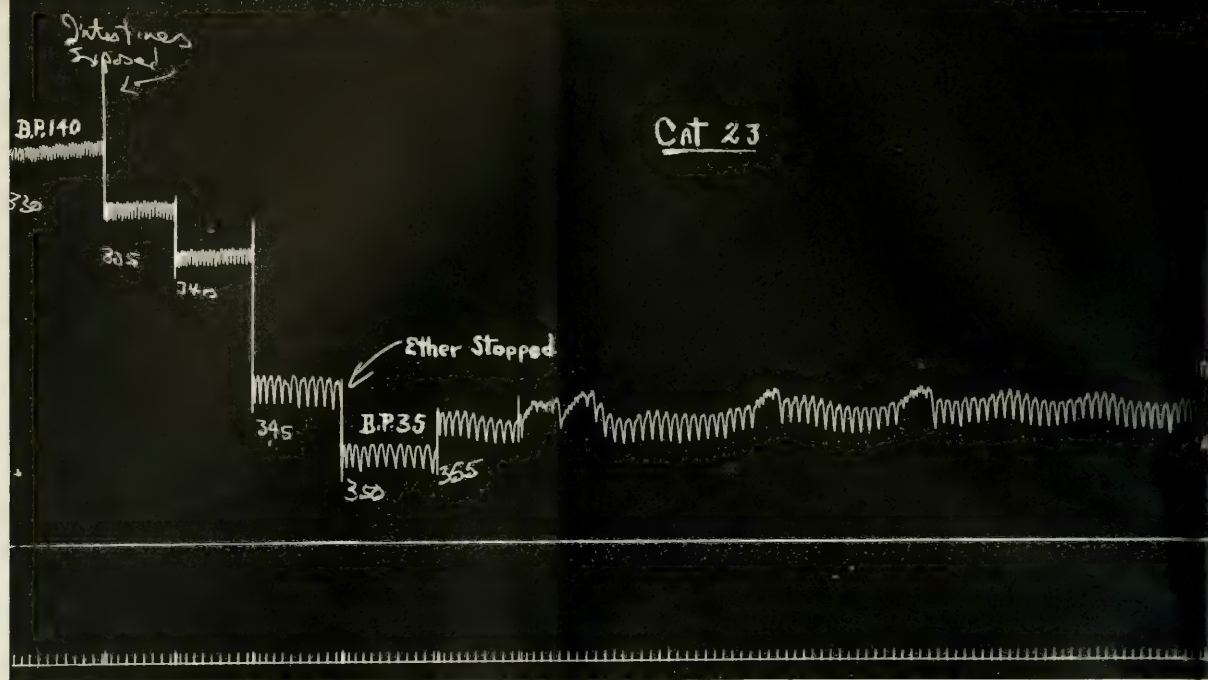


FIG. 1.—Careless anesthesia resulting in condition resembling shock.

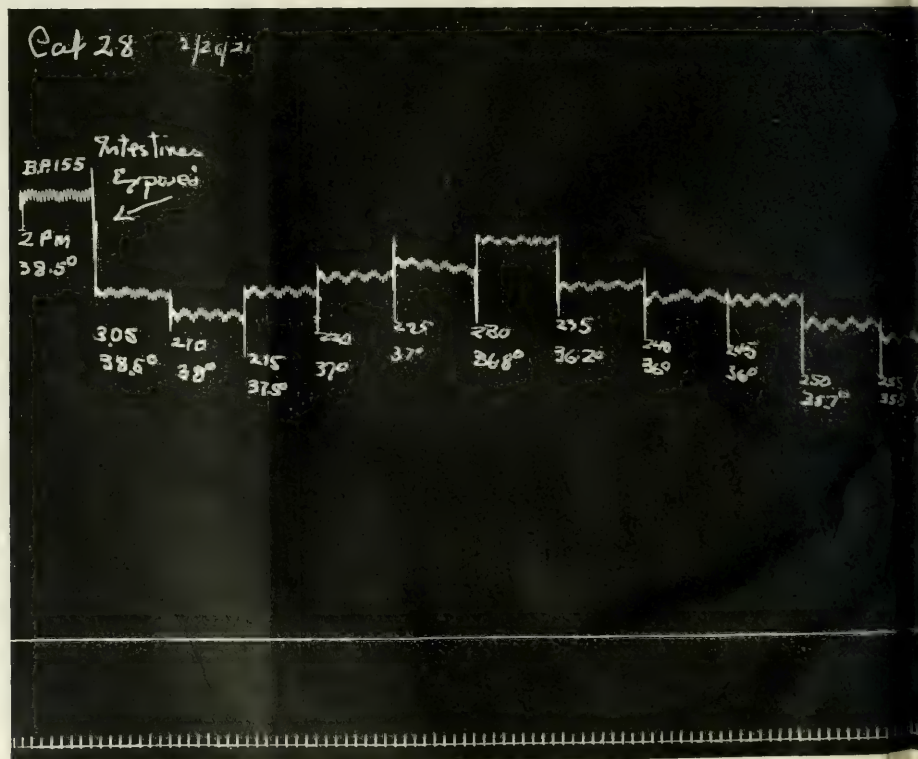


FIG. 2.—Blood pressure tracing of normal animal during production of shock by intestinal exposure. As pressure falls, a not infrequent occurrence.

their appearance. The time required for the development of complete shock in these 10 animals was remarkably constant, varying between the extremes of an hour and a half and two hours, the average time being an hour and 50 minutes. Fig. 2 represents a typical experiment from this group.

Reaction of Animals Immediately Following Adrenalectomy.—Having determined the time required to bring normal animals into shock, we next carried out a series of experiments to study the reaction of adrenalectomized animals subjected to the same conditions. Each animal was anesthetized, tracheotomized and a blood pressure tracing taken just as in the first series. Then both adrenals were carefully removed extraperitoneally, and the lumbar incisions were closed with sutures. This procedure required 30 or 40 minutes and was performed with no more hemorrhage than would stain part of a small piece of gauze. Kymographic tracings were taken every five minutes for about 20 minutes following removal of the glands in order to observe the immediate effect of the operation. During this time the blood pressure invariably either remained at the original level or, if anything, occasionally rose a few millimeters above it. The intestines were then exposed and manipulated exactly as in the normal controls, the method of anesthesia being the same throughout.

The reaction of these animals was most interesting. Kymographic tracings taken every five minutes, as before, showed that there occurred the usual slight fall of blood pressure immediately following the opening of the abdomen. This fall was promptly recovered from in several experiments; but in every experiment, during observations continued as long as two and a half and three hours following the exposure of the intestines, the blood pressure exhibited no further fall than the negligible depression which prolonged light anesthesia alone produces; and cessation of the anesthetic at the end of this time was invariably followed by prompt and complete recovery, although these animals were subjected to exactly the same conditions as the normal controls which were in deep shock, with the blood pressure at 60 millimeters or below, after only an hour and 50 minutes of intestinal manipulation. Fig. 3 is typical of this series. It will be seen that the blood pressure at the end of two hours and a half of intestinal manipulation stands at 110 millimeters, and has fallen from the original level no more than the blood pressure of normal animals kept quietly under ether anesthesia for the same length of time. The pulse is of splendid quality; and when the anesthetic was discontinued the animal promptly recovered full consciousness and sensibility. Not one of these 10 animals went into shock. Autopsy showed in every case complete removal of both adrenals.

This series of experiments seemed to favor strongly the idea that shock might be the result of some abnormal secretion of the adrenals, since there appeared no tendency for the condition to develop in adrenalectomized animals subjected to the same conditions which regularly produced it in the normal animal. There were, however, aside from the lack of adrenals, two points in which the adrenalectomized animals differed from the control animals at the beginning of the intestinal ex-

posure—they had undergone an operation, and they had been kept under anesthesia for about an hour during the operation and the subsequent blood pressure observations made before the abdomen was opened. The fact that they had been subjected to an operation before beginning the shock trauma would be expected to increase their tendency to shock, if anything, and so too would a preliminary hour's anesthesia. A third series of experiments was carried out, however, to study the reaction of animals to intestinal manipulation following a preliminary hour's anesthesia alone.

Reaction After a Preliminary Hour's Anesthesia.—After tracheotomy and measurement of the blood pressure, the animals of this series were kept quietly under light anesthesia for an hour, kymographic tracings being taken every five minutes. Then the intestines were exposed and handled just as before. These animals reacted precisely as those of the adrenalectomized series; they showed no tendency to fall into shock during three hours of intestinal manipulation. Fig. 4 is a tracing from a typical experiment. The blood pressure shows no more tendency to fall after intestinal exposure than if the animal were merely kept anesthetized for the same length of time. Two hours and a half after the abdomen was opened the systolic pressure was 120 millimeters, the pulse was good, and when the anesthetic was discontinued, the animal regained full consciousness and sensibility and had to be killed. It will be noted that, in this particular experiment, besides the usual manipulation of the intestines every five minutes, considerable further trauma was inflicted without effect. The kidneys were roughly handled, several inches of intestine were resected at intervals, and the parietal and visceral peritoneum was stretched severely, with only a slight temporary effect upon the blood pressure.

In order to be certain that the resistance of these animals to shock was brought about by the preliminary hour's anesthesia rather than by some unrecognized technical divergence from the method used in the normal controls, two control experiments were now repeated, the intestines being exposed and handled immediately following the first blood pressure reading, instead of after an hour's anesthesia. These animals behaved exactly as the original normal controls. They were in deep shock in an hour and 55 minutes and two hours, respectively.

The 12 experiments with preliminary anesthesia were clear-cut, and demonstrated that an animal which has been kept anesthetized with ether for an hour immediately before opening the abdomen becomes, in some way, much more resistant to the shock-producing effects of intestinal manipulation than are animals in which the intestines are exposed more promptly after anesthetization. No explanation of the mechanism of this protective ether-effect can be offered here other than the suggestion that during the early stages of anesthesia the circulation appears to be in a rather unstable condition, and the added strain of visceral trauma at this period can, perhaps, bring about circulatory failure more easily than in an animal which has had time for its circulation to become accommodated to the state of anesthesia, and more stabilized. It is note-

worthy, however, that although an hour's light anesthesia *before* opening the abdomen had this protective effect upon the circulation, in other experiments it was clear that, once the blood pressure had begun to fall toward the shock level after intestinal manipulation had been begun, ether exerted a marked depressant action upon the circulation and distinctly favored the development of shock.

It is evident that no conclusions regarding the relation of the adrenals to shock could be drawn from the experiments in which the glands were removed immediately before exposing the intestines. The complicating factor of the protective ether-effect precluded that. It was, therefore, decided to remove the adrenals, to allow the animal to recover completely from the anesthetic, then to reanesthetize it afresh and proceed at once with the intestinal trauma.

Reaction Seven to Seventeen Hours after Adrenalectomy.—In this series of experiments both adrenals were removed extraperitoneally through lumbar incisions under strict aseptic precautions. The animals were then allowed to recover from the anesthetic and were left in their cages for periods ranging between 7 and 17 hours. None of the animals displayed any sign of adrenal insufficiency on inspection. They appeared active and not asthenic. Each animal was then anesthetized, tracheotomized, a blood pressure tracing taken and the intestines at once exposed and handled as usual. It was found at the outset that these adrenalectomized animals had a very low blood pressure, the first readings averaging 82 millimeters. The pulse was more rapid than normal. All of the animals of this series fell into deep shock within 30 or 35 minutes after exposure of the intestines, the blood pressure falling rapidly to 20 millimeters in most cases. At autopsy the operative sites were found in splendid condition, and dissection of the splanchnic nerves showed injury only to the small branches which necessarily must be severed in removing the adrenals.

Fig. 5 is a tracing from a typical experiment in this series.

Although these adrenalectomized animals went into shock in one-third the time required to bring a normal animal into shock, it was evident from the low blood pressure and rapid pulse that the circulation was already affected by the removal of the adrenals before intestinal manipulation was begun. It was not possible, therefore, to draw conclusions from this series concerning the relation of the adrenals to shock, and it was realized that experiments would have to be carried out upon adrenalectomized animals which had been allowed to recover from the anesthetic after adrenalectomy, in order to eliminate the protective ether-effect, but which had not been left long enough for circulatory signs of adrenal deficiency to appear before the animals were exposed to the shock-trauma.

Reaction One Hour After Adrenalectomy.—Since the blood pressure does not begin to decline until several hours after adrenalectomy, a series of experiments was carried out in which, following removal of both adrenals under aseptic precautions, each animal was allowed to recover fully from the anesthetic until it was able to walk about and to react normally to stimuli. In this way the protective effect of the anesthesia administered during the operation was avoided, since it had been determined

in two experiments that if an animal were reanesthetized immediately after recovery from an hour's anesthesia, it retained none of the protective effect of the ether, but reacted to intestinal manipulation exactly as a normal animal. Complete recovery from the anesthetic administered during adrenalectomy required usually about 30 minutes; then the animal was reanesthetized at once, tracheotomized, and a blood pressure tracing made. The blood pressure was normal in every case, averaging 145 millimeters. The intestines were then exposed at once and handled as usual. These adrenalectomized animals reacted exactly as normal animals, requiring an average of an hour and 45 minutes to fall into shock. Fig. 6 is a typical tracing from this group.

This series of experiments indicates clearly that shock develops in the absence of the adrenal glands precisely and characteristically as it does in normal animals. Disordered function of the adrenals cannot be invoked as the cause of shock produced by peritoneal trauma. If the adrenals were causative factors of any importance, there would have appeared some difference between the reaction of the adrenalectomized animals and that of the normal controls. It might be objected that perhaps some of the secretion of the adrenals remains active in the body for a while after the glands are removed, so that adrenalectomy does not create an immediate deficiency of the secretion. It must be clear, however, that the same objection would be more applicable to any theory postulating disordered adrenal function as a cause of shock. The importance of the accessory chromaffin tissue is recognized fully, but since the adrenalectomized animals enter the experiment with complete absence of the adrenal glands, surely if epinephrin exhaustion or adrenal fatigue were causative factors in shock, these animals should fall into shock distinctly quicker than normal animals in which a longer time would be necessary to exhaust the greater epinephrin supply or bring the adrenal glands as well as the accessory tissue into a condition of dysfunction.

Condition of the Heart and Medullary Centers in Shock.—

During the progress of these experiments advantage was taken of the opportunities to study the condition of various body functions during shock. Most observers have found that the heart functions normally during shock. Erlanger and his co-workers²⁰ acquired the impression that the heart in shock has not the normal reserve power and this might be expected, since Markwald and Starling have shown that weakening of the cardiac contraction accompanies low blood pressure.²¹ Boice²² attributed the circulatory failure in shock to impairment of cardiac function. In the present experiments the heart during shock has invariably worked splendidly both under the strain of large doses of adrenalin, and when normal saline was run rapidly into the jugular vein. In every experiment in which an animal was allowed to die in shock, respiratory rather than cardiac failure was the immediate cause of death.

It is almost universally stated that the pulse is rapid during shock. Wiggers, however, mentions slowing of the heart during the late stages of the condition²³ and Dupuytren²⁴ wrote from clinical observations "Le pouls est d'une lenteur et d'une

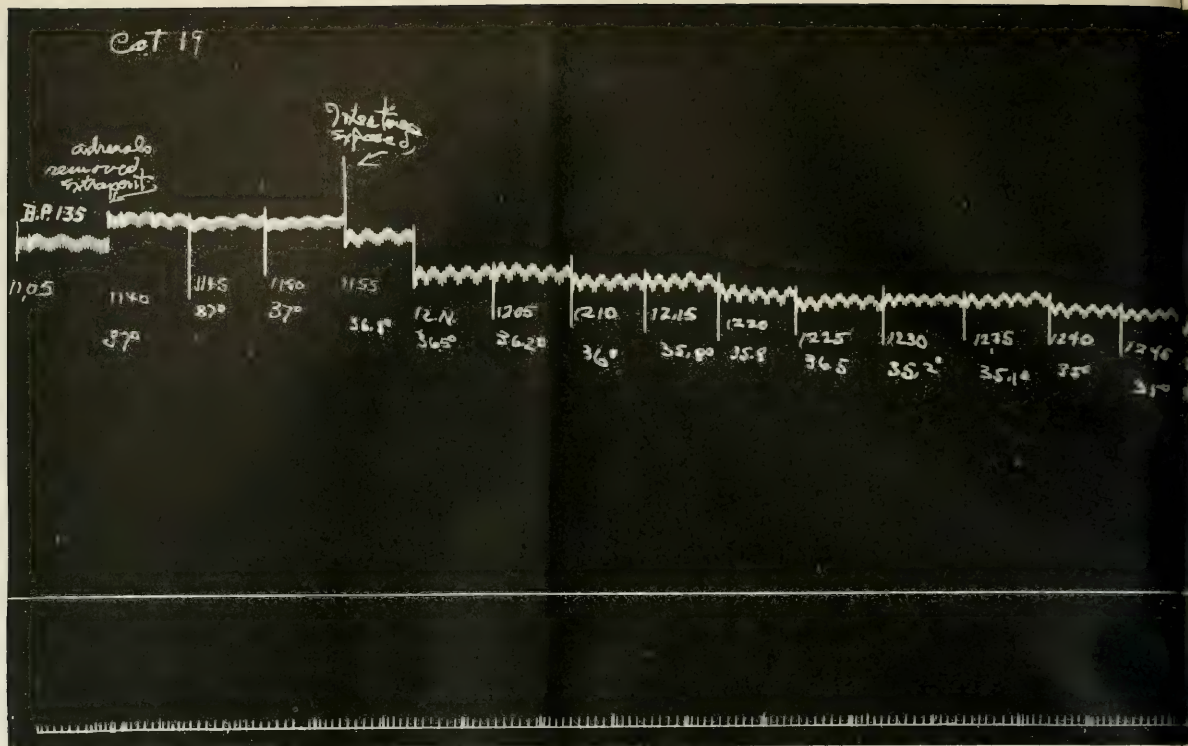


FIG. 3.—Adrenals removed at 11.40 a. m.; intestinal manipulation begun at 11.55 a. m.

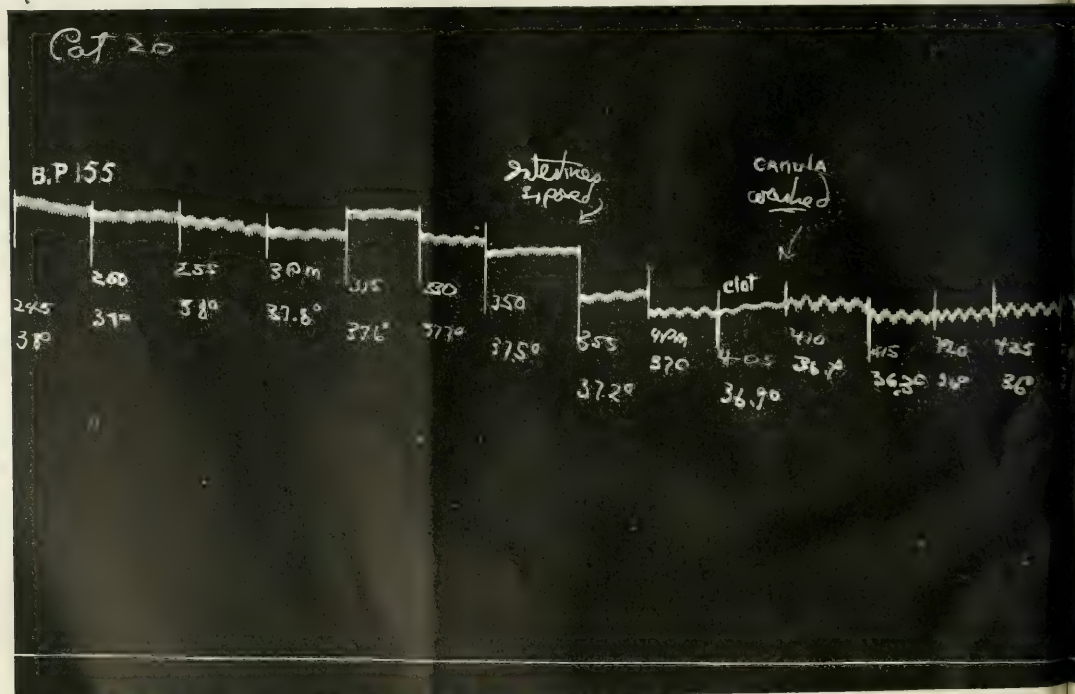
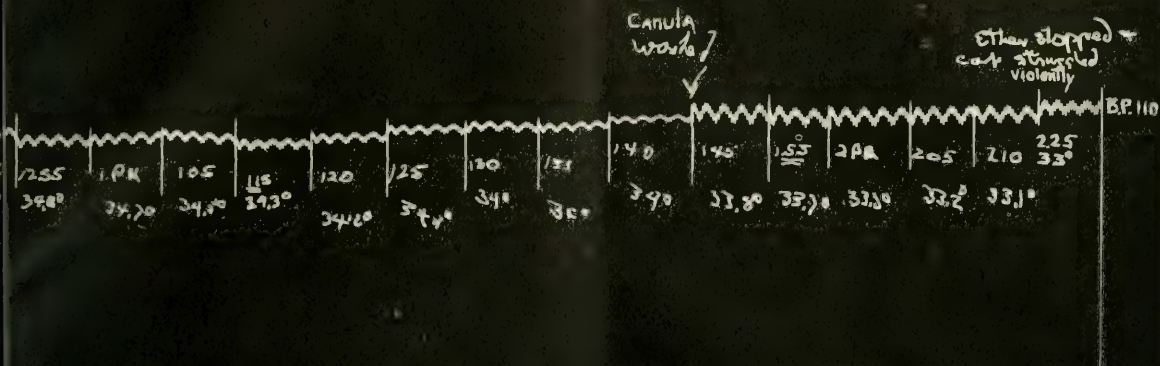
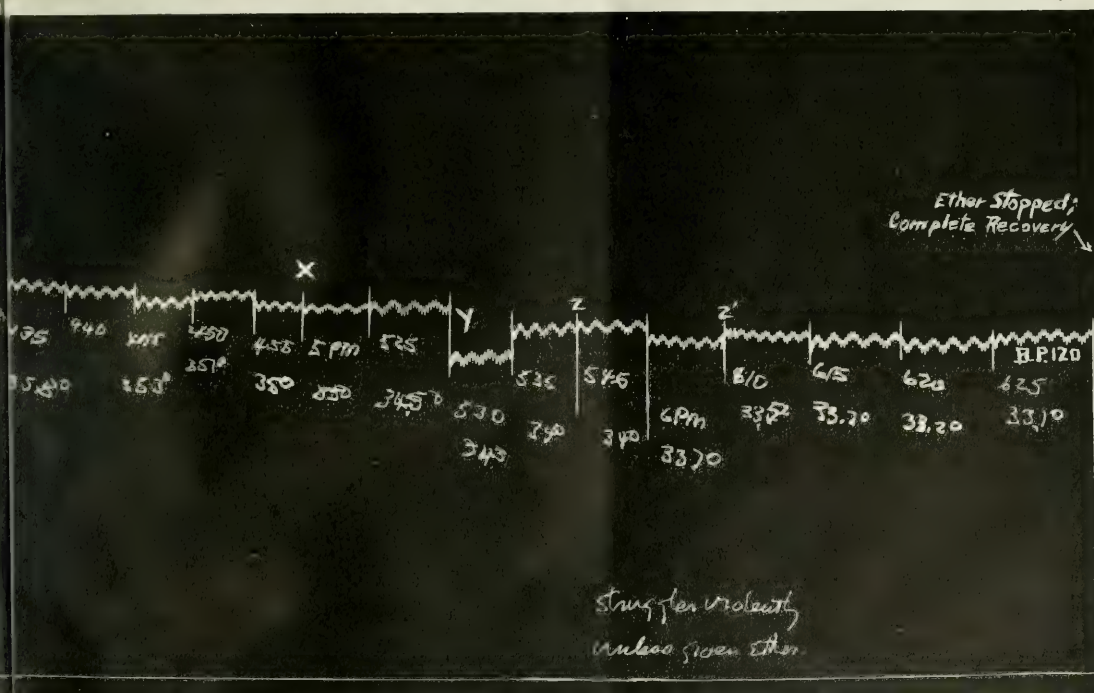


FIG. 4.—Intestinal manipulation after an hour's preliminary anesthesia. Besides the usual manipulation every 5 minutes, severe traction was applied at 3.55 p. m. Condition of animal normal.



Condition of animal normal after 2½ hours of intestinal trauma. No shock.



peritoneum and mesentery was made at X; kidneys roughly handled at Y; resection of several inches of intestine at Z and Z'.
 at 2½ hours. No shock.

mollesse telles que la plus légère pression le sufflamine," and that, if recovery takes place, "le pouls est plus fort et moins rare." In a number of the present experiments periods of pronounced slowing of the pulse were observed during deep shock. This slowing of the heart-beat is a result of the action of the cardio-inhibitory center. It has been held that inhibition or paralysis of this center is a characteristic and perhaps a cause of shock; on the other hand, the experiments of numerous workers indicate that the cardio-inhibitory center functions normally during shock. Mann reported that stimulation of the central end of one cut vagus caused reflex cardiac inhibition "even in the most extreme degrees of shock."²¹ Jackson and Ewing wrote that they were able to obtain the reflex only while the blood pressure remained above 60 millimeters of mercury.²² I have never failed to obtain slowing of the heart-beat during shock by this means, and the reflex has been elicited repeatedly when the blood pressure was as low as 20 millimeters (Fig. 7). In these experiments a further proof that the heart, in shock, is under control of the cardio-inhibitory center was obtained by observing the effect of cutting the vagi. If the vagi are sectioned during one of the periods of bradycardia which occur occasionally when an animal is in deep shock, a most striking acceleration of the pulse always results immediately, as can be seen in Fig. 8, where the heart-rate increased from 80 to 200 per minute. Section of the vagi when the pulse is rapid during shock will always increase the heart-rate unless it is already too rapid to be influenced by removal of vagus inhibition; and in the cases in which vagus section could not increase the rate, stimulation of the central end of one cut vagus has already produced slowing of the heart immediately before section of the remaining vagus, showing that the cardio-inhibitory center was capable of responding to stimuli during shock. In three experiments both vagi were sectioned before exposing the intestines; these animals went into shock no more rapidly than the normal controls, and their blood pressure curves were characteristic of the normal shock curves. It is clear that failure of the cardio-inhibitory center is not a causative factor in shock.

The question of the condition of the vasomotor center has been the subject of many investigations. On the basis of their well-known experiments, Crile,²⁴ Lockhart-Mummery²⁵ and others have maintained vigorously that surgical shock represents an exhaustion of the vasomotor center; others^{26, 27, 28, 29} have produced splendid evidence that it is not exhausted. In the present experiment, a pressor response could always be elicited by stimulation of sensory nerves during shock. In most of the experiments the rise of blood pressure was less prompt and less marked than before the onset of shock, although in some cases the rise was quite as marked. Fig. 9 shows a definite pressor response obtained by sciatic stimulation during deep shock, when the blood pressure was only 22 millimeters. I can entirely agree with those who hold that exhaustion of the vasomotor center is not a primary factor in shock.

Janeway and Ewing¹⁷ have stated that in shock produced by intestinal manipulation "there is absolute paralysis of every

tissue of the intestines, of the muscles, of the intestinal walls and of the arterioles," and they stress the importance of a local peripheral paralysis of the splanchnics as a factor in shock. I have never observed such a complete paralysis of the intestines during shock. Abortive peristaltic movements occur throughout the duration of shock, and not infrequently persist for a short while after death. Janeway and Ewing wrote that in some of their experiments the intestinal manipulation was quite violent, causing rhexis of the peritoneum. It is possible that excessive trauma might produce such a paralysis, but surely it cannot be regarded as a characteristic part of shock.

The Hypotension Resulting from Adrenalectomy.—It may not be entirely out of place here to describe several observations upon the group of adrenalectomized animals which may have some bearing upon the function of the adrenal glands.

In the first place, although we have the clinical evidence of Addison's disease and certain experimental evidence besides, indicating some relation of the adrenal glands to the maintenance of normal blood pressure, Vincent³⁰ reports that continuous blood pressure tracings, taken up to the moment of death, after complete removal of the adrenals from the circulation, show no greater fall in blood pressure than would have occurred in a normal animal subjected to ether anesthesia alone for the same period of time, and he concludes that "these experiments appear to show conclusively that the secretion of adrenalin into the circulation is not to be regarded as a factor in the maintenance of the normal blood pressure." The present experiments do not at all agree with such an observation. Howell³¹ and others have pointed out that adrenalectomy has no effect upon the blood pressure for several hours, and I have observed this fact repeatedly. But the blood pressure of an adrenalectomized cat begins to fall gradually about four hours after the glands have been removed, and within the first 12 hours after adrenalectomy the blood pressure in the unanesthetized animal invariably falls to a very low level and continues to decline progressively until death. In these experiments it was demonstrated that the marked hypotension, observed in every case as early as seven hours after adrenalectomy, is a characteristic result of adrenal deficiency and not merely a condition of lowered tone in an animal several hours after an operation. For in a number of experiments both adrenals were exposed aseptically and handled even longer than would have been necessary to remove them. They were then left in their normal positions and the incisions closed. Seven to twelve hours later these animals were reanesthetized and the blood pressure in every case was normal and exposure of their intestines produced shock in the length of time characteristic of normal animals. In another series, under aseptic precautions, one adrenal was removed and the other exposed and handled, the operation taking as long as if both had been removed and the operative trauma being made purposely more severe. When these animals were reanesthetized after a lapse of from 7 to 15 hours, their blood pressure was found to be normal and they reacted to intestinal manipulation exactly as normal controls. Autopsy showed complete removal of one

adrenal in every case. Thus, the trauma of the operation alone is not responsible for the low blood pressure which developed in every completely adrenalectomized animal; nor has removal of one adrenal any effect upon the blood pressure during the period of these experiments; but when both glands are removed the blood pressure invariably falls strikingly. The splanchnic nerves were dissected at autopsy in every case in order to make certain that injury was confined only to the little branches which must be cut in order to remove the adrenals. I cannot agree with those who have claimed that the low blood pressure developing after adrenalectomy is merely the hypotension to be observed in any moribund animal, nor does the contention of Hoskins and McClure,¹⁴ that the hypotension is a result of asthenia, seem reasonable; for these adrenalectomized animals were by no means in a moribund condition, nor was there evident asthenia seven hours after removal of the glands. One of these adrenalectomized animals, a rather wild male, escaped from its cage just before it was anesthetized for the blood pressure reading and ran swiftly about the room, evading capture. It was caught, anesthetized, and the systolic pressure found to be 75. It seems more reasonable to believe that the hypotension leads finally to asthenia because of the impairment of the circulation.

The fact that hypotension is a characteristic effect of adrenalectomy and that it appears to be the result neither of the trauma of the operation nor of the asthenia, supports the belief that the adrenals are, after all, directly concerned in some way in the maintenance of normal blood pressure. This idea is strengthened by observations upon the powers of resistance of the circulation of adrenalectomized animals to strains (such as prolonged anesthesia and trauma) imposed upon it. These experiments are as yet incomplete, but they strongly suggest that the circulation of an adrenalectomized animal, after the development of hypotension but before the appearance of asthenia, is distinctly more unstable than that of a non-adrenalectomized animal with the same degree of hypotension.

DISCUSSION

It may possibly appear inconsistent that any observations supporting the belief of the activity of the adrenals in maintaining normal blood pressure are presented here together with evidence that these glands are not concerned in the circulatory failure characteristic of shock; but certainly, even though the adrenals be concerned with blood pressure regulation, they are not by any means the only factor involved. The demonstration of the lack of any causal relation between the adrenals and the acute hypotension of shock merely points more distinctly to a disturbance of some other part of the intricate circulatory mechanism.

At the present time, the idea that the adrenals may be concerned with the maintenance of normal blood pressure is widely repudiated, and the work of Cannon, Gley, Stewart and Rogoff, Hoskins and McClure, and Vincent, in this connection is familiar enough to everyone who has given any attention to the function of these glands. The objection has been repeatedly brought forth that if adrenalin were necessary for the

maintenance of normal blood pressure, removal of the glands should result in an *immediate* fall of pressure; but no fall occurs during several hours after adrenalectomy.^{14, 23, 25, 27} Much has been made, also, of the fact that adrenalin can be detected in normal blood in minute amounts only; so minute, indeed, that numerous writers do not hesitate to declare that it is physiologically useless in affecting vasomotor tone, especially since the intravenous injection of small amounts of adrenalin can produce a depressor instead of a pressor effect.^{15, 25, 26, 28} Perhaps these, and certain other similar observations which cannot be discussed here, deserve more critical consideration and study before they can be accepted as reasons for believing that adrenalin plays no part in the maintenance of normal arterial pressure, or that "l'adrénaline ne doit plus être considérée comme un produit de sécrétion vraie."²⁵

In regard to the objection that the blood pressure does not fall *immediately* after adrenalectomy (and some have actually been content with blood pressure observations continued less than two minutes after compression of the adrenal veins)²⁷ it does not seem permissible to ignore completely the fact that there is a definite supply of physiologically active epinephrin left in the accessory chromaffin tissue after removal of the adrenal glands,^{29, 30} and that this supply may be sufficient to sustain the blood pressure for several hours, but not indefinitely. It is true that Stewart and Rogoff have considered this point to the extent of writing: "No account is here taken of the possibility that the sporadic chromaffin tissue may discharge a certain amount of epinephrin, since, although it has been shown to contain that substance, nothing is known as to its liberation";²⁹ however, the fact of our ignorance as to the mode of liberation of epinephrin from this accessory chromaffin tissue is not a satisfactory reason for taking no account of it. The well known protective effect of minute residual fragments of parathyroid, "thyroid and adrenal" tissue left, either intentionally or by accident, in extirpation experiments demonstrates strikingly enough the rôle that a relatively insignificant amount of such tissue can play. It is curious, in any event, that those who feel that the adrenals have nothing directly to do with vascular tone, because hypotension does not occur the instant the adrenal veins are clamped, nevertheless appear to accept the belief that the glands are concerned in some way with the maintenance of muscular tone, although asthenia develops, after adrenalectomy, distinctly later than hypotension. It seems to the writer that the important fact may be, not that the blood pressure fails to fall immediately after removal of the glands, but rather that a primary effect of adrenalectomy is hypotension.

Concerning the minute amount of epinephrin that can be detected in the blood, it is difficult to be sure that it has no physiological significance in relation to normal blood pressure simply because the amount collected at any given moment is too small to affect the contractions of a strip of smooth muscle *in vitro*, or to exert a pressor effect upon the blood pressure when introduced into the circulation of an animal. Unquestionably, such procedures appear to be surprisingly delicate quantitative tests for the presence of epinephrin within wide

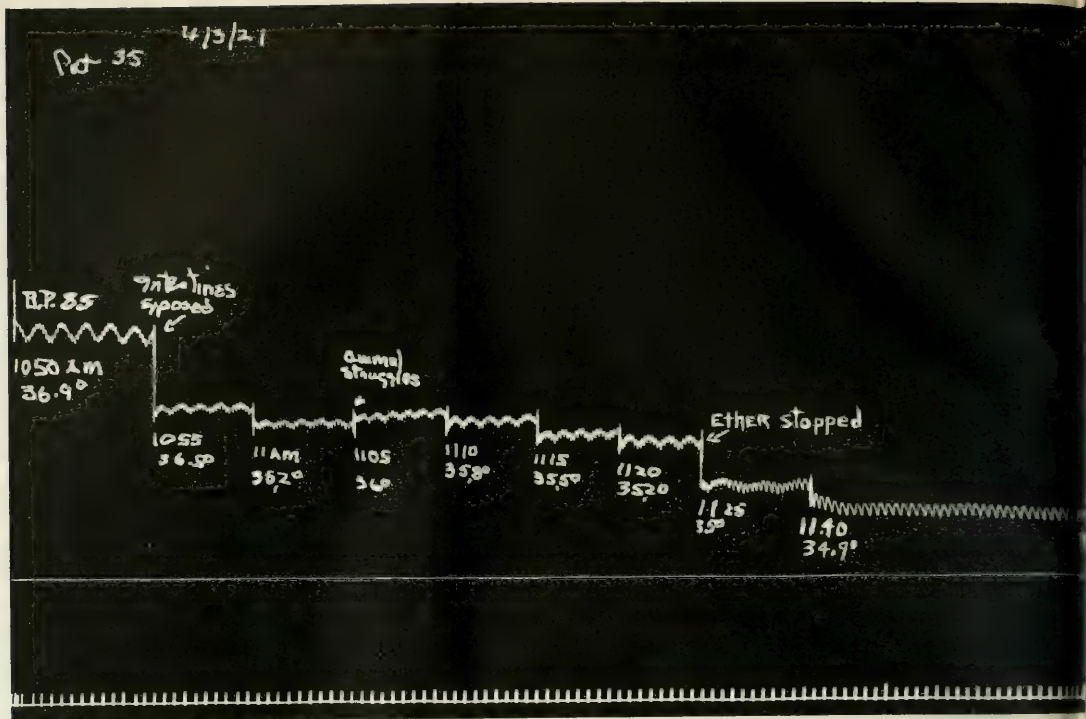


FIG. 5.—Intestinal manipulation 11 hours after complete adrenalectomy. April 2, 11.50 p. m., both adrenals removed; April 3, 10.40 a. m., about, apparently normal. Intestines exposed 10.55. Deep shock 30 minutes later.

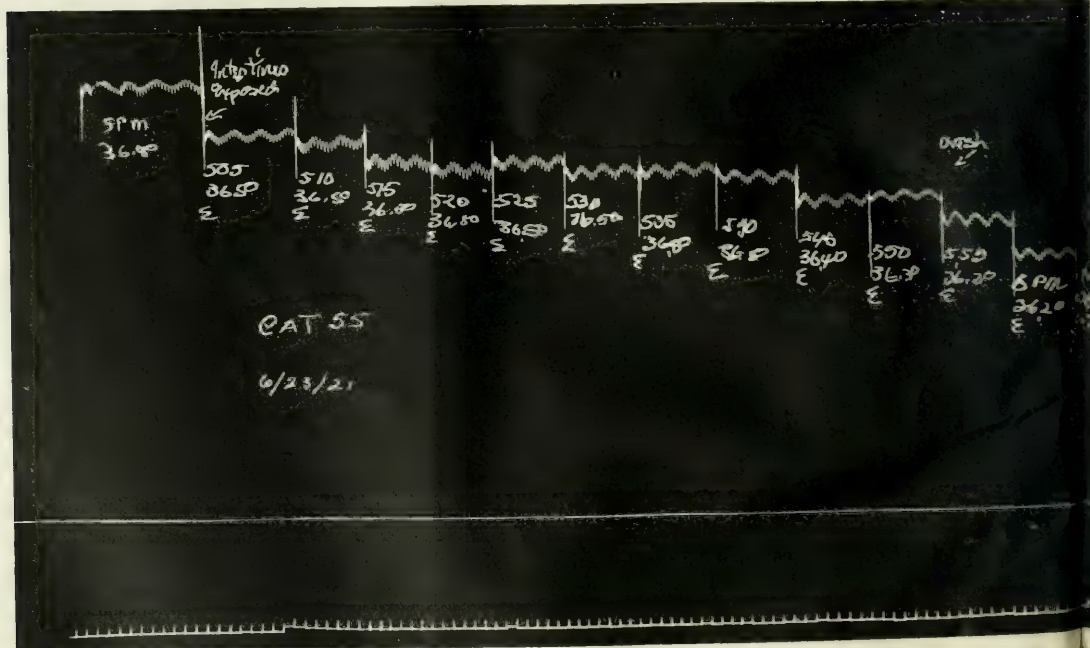


FIG. 6.—Intestinal manipulation 1 hour after adrenalectomy. June 23, 3.30 p. m., operation for removal of both adrenals begun; off table at 5 PM.

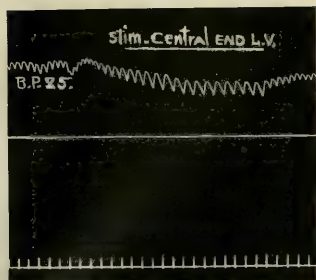


FIG. 7.—Slowing of the heart produced during deep shock by stimulating central end of cut left vagus (B. P. 25).

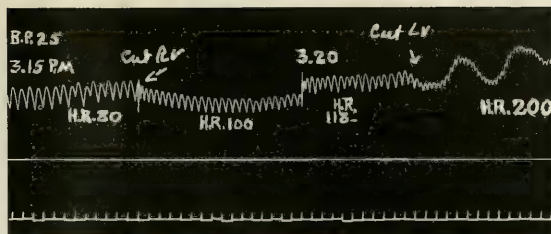


FIG. 8.—Shows activity of cardio-inhibitory center producing spontaneous bradycardia during deep shock. Heart rate increases from 80 to 200 per minute on section of vagi.

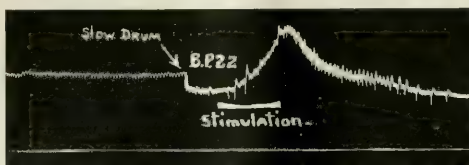
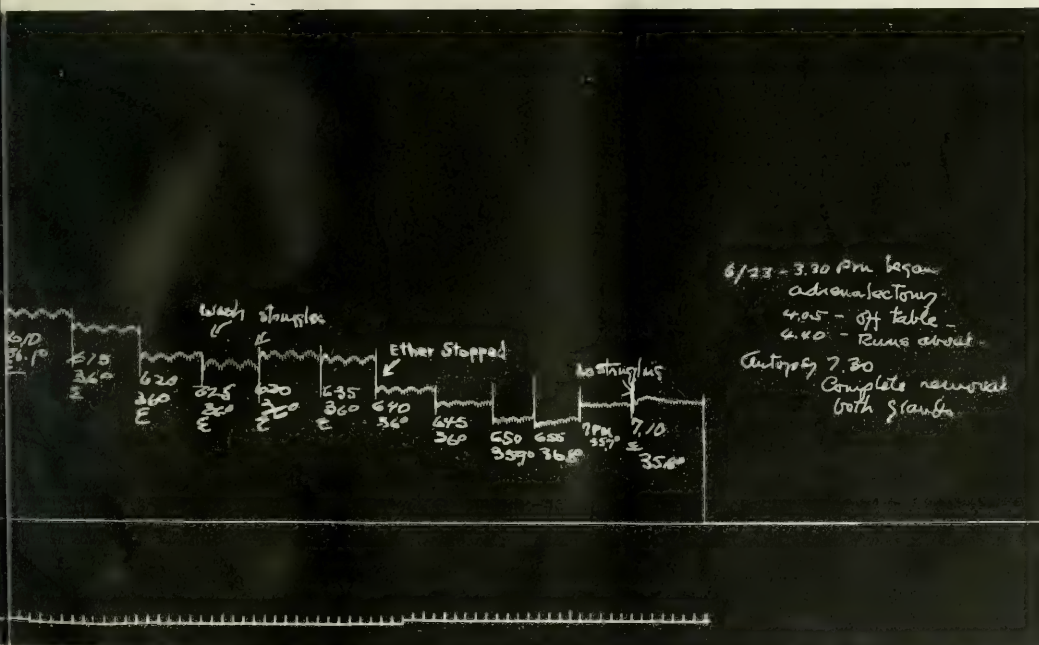


FIG. 9.—Pressor response elicited by stimulation of sciatic nerve during deep shock (B. P. 22).



Complete recovery from anesthetic at 4.40 p. m. Intestines exposed at 5.05 p. m. Shock 1 hour 35 minutes later. Compare with Fig. 2.

limits; however, they have limitations, a point which Stewart and Rogoff did not ignore when they concluded from certain experiments that the "liberation of epinephrin from the adrenals is not indispensable for life or health, unless indeed the necessary quantity is, even in the adrenal vein blood, below the limits of detection by the methods used."²⁸ The fact that the quantity of epinephrin in the blood, at any given time, is minute or even undetectable by present methods (Stewart and Rogoff, however, do detect it unfailingly in normal adrenal vein blood) does not entirely satisfy the question of the relation of this substance to normal blood pressure. In the first place, the methods of collecting and assaying the blood samples and the conditions under which the blood is obtained are apparently so subject to error that almost each worker has devoted some space in the literature to a more or less caustic criticism of the other's technique: and in the second place, it is highly probable that there may be a difference between the physiological potentialities of minute amounts of a substance secreted continually, and the effects which can be demonstrated when the amount that is withdrawn from the circulation at any one cross-section of time is used in an acute experiment. Otherwise, we would have to conclude from a study of small samples of normal arterial blood that the secretion of the thyroid gland has no effect upon body oxidations, and that of the para-thyroids no relation to calcium metabolism.

The most elaborate and important recent work on the adrenals^{4, 22, 29, 30} has been concentrated upon the question of the existence of a physiological adrenalinaemia, and has been concerned not at all with the fact that the most striking primary effect of the lack of the adrenal secretion is hypotension. Unquestionably, the painstaking and ingenious experiments of these workers hold a very great interest. Because of the ease with which epinephrin can be detected, the study of the epinephrin content of the blood promised much, and it was most important that it be carried out. However, it is perhaps permissible to feel that this study, even in the hands of such able investigators, has not led to conclusions as concordant or decisive as one might have hoped for. It must be remembered that there still exists an active controversy between these workers over the amount of epinephrin normally present in the blood, and also over the more important question of whether the epinephrin content of the blood is increased during the various reflex pressor reactions which have been assumed to be conditioned or influenced by an outpouring of this secretion. The only point of agreement, in fact, seems to be the opinion that the amount of epinephrin circulating normally is not great enough to exert an influence upon normal blood pressure. It is quite possible that epinephrin has nothing to do with the maintenance of normal pressure, as these observers insist; it may, indeed, be that the lack of the unknown cortical secretion is responsible in some way for the hypotension that follows adrenalectomy. Nevertheless, the observations leading to a denial of any relation of epinephrin to normal arterial pressure have certainly not yet settled this important question, which must remain incompletely answered until we are able to understand why the blood pressure falls

so characteristically when the adrenal glands are removed from the body.

SUMMARY

I. Adrenalectomized animals, subjected to uniform intestinal manipulation before the blood pressure has begun to decline as a result of adrenalectomy, fall into shock exactly as do normal controls—the time required for the production of shock and the character of the blood pressure curves being the same in both series. It is therefore concluded that disordered adrenal function is not a factor in the production of shock.

II. Hypotension invariably results from removal of the adrenal glands, and with the development of hypotension the circulation of adrenalectomized animals appears to become more unstable than that of normal animals even before the appearance of asthenia. The blood pressure begins to fall several hours after adrenalectomy and becomes progressively lower until death. The fall in blood pressure is shown to be independent of the operative trauma and begins before asthenia has appeared. This is offered in support of the idea that the adrenals are concerned in the maintenance of the blood pressure at the normal level, and certain objections to this belief are briefly discussed.

III. Animals that are kept lightly anesthetized with ether, for an hour immediately before the abdomen is opened, become very resistant to the shock-producing effect of intestinal manipulation. Even when subjected to severe peritoneal trauma for a period of three hours, the blood pressure shows practically no tendency to fall and sensibility is retained. In contrast, if identical intestinal manipulation is begun more promptly after anesthetization, the blood pressure invariably begins to decline progressively within an hour, has fallen to 60 mm. or below an hour and a half to two hours after opening the abdomen and the animal is in complete shock. An hour's ether anesthesia preliminary to opening the abdomen has proved to be a striking protective against shock, under the conditions of these experiments. If an animal is kept anesthetized for an hour, permitted to recover from the anesthetic, and at once reanesthetized and intestinal manipulation begun, the protective effect of the hour's anesthesia will have disappeared.

IV. Ether has a distinct tendency to hasten the onset of shock once the blood pressure has begun to decline after the abdomen is opened.

V. Cardiac failure is not a factor in the production of shock.

VI. Failure of the vasomotor center is not a primary factor in shock.

VII. The cardio-inhibitory center is shown not only to respond to stimuli but also to function independently during deep shock. Its failure cannot be regarded as a cause of the condition.

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A CLINICAL AND ANATOMICAL STUDY OF FIFTY-ONE CASES OF REPEATED CÆSAREAN SECTION WITH ESPECIAL REFERENCE TO THE HEALING OF THE CICATRIX AND TO THE OCCURRENCE OF RUPTURE THROUGH IT

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In 1917 Dr. J. Whitridge Williams published in the BULLETIN OF THE JOHNS HOPKINS HOSPITAL the results of a histological study of 50 uteri removed at Cæsarean section. Included among them were 10 uteri which had been incised at a previous similar operation. They were only briefly described, as Dr. Williams stated that "the details concerning this series will be published in full later." Since that time 11 additional specimens have been added to our collection, and it is my purpose to report the results of a careful clinical and anatomical study of this material.

It is but a natural sequence that, while investigating the histories of the women from whom the uteri were removed, we should likewise consider the closely allied cases in which a conservative Cæsarean section was repeated at a subsequent pregnancy, or delivery was effected through the natural passages. It is only by a careful consideration of such cases that we may be enabled to draw definite conclusions concerning the truth or fallacy of the oft-quoted dictum—"Once a Cæsarean, always a Cæsarean."

Our discussion is based upon the study of 63 pregnancies occurring in 51 women who had previously been subjected to Cæsarean section. Fifty-five of these pregnancies followed a single Cæsarean section, while in eight there were two opera-

tions. The manner in which the pregnancies were terminated was as follows:

A second or third Cæsarean section was done in 45 cases.

Vaginal delivery occurred in 17 cases.

Rupture of the old Cæsarean scar occurred in one case.

That the woman who has once been subjected to a Cæsarean section, and who again becomes pregnant, presents an interesting and at the same time a somewhat puzzling problem has been generally recognized and frequently commented upon. Prior to 1876 the mortality following Cæsarean section was appalling, from 50 to 60 per cent of the women dying from infection or hæmorrhage, but at the present day elective Cæsarean section, performed under proper surroundings by men trained to recognize its indications, as well as its contraindications, should be attended by an immediate mortality of not more than one per cent. From an obstetrical point of view we cannot, with fairness, rest content with the immediate results, but must also consider what may happen in the subsequent pregnancies of such women. Rongy has estimated that approximately three per cent of all Cæsarean section scars eventually rupture, and that at least one-half of such accidents terminate fatally. Notwithstanding this very definite menace, and completely ignoring the frequent warnings, which have been sounded by many writers in recent years, surgeons, gynecolo-

gists, and obstetricians throughout the country are performing the operation with increasing frequency.

Proof that the uterine scar constitutes a *locus minoris resistentiae* in a certain proportion of cases may be obtained by reviewing the literature on rupture of the Cæsarean scar in subsequent pregnancies. Approximately 102 such cases have been reported and summarized by numerous authors. Thus, Wyss, in 1912, analyzed 42 cases, while four years later Bell, Schroeder, and Findley collected 79, 63, and 63 cases, respectively. To the 63 cases reported by the latter, Spalding, in 1917, added 12 others, which he had collected from the literature. Furthermore, Losee reported nine instances of partial or complete rupture in 1918, and since then Davis, DeCoursey, Howson, Freund, Novak, Baisch and Holland have reported additional cases. In addition to the occurrence of actual rupture, a number of cases have been reported in which the uterine scar was so attenuated that rupture would probably have occurred had the uterine distention not been relieved by operation, not to speak of those in which the rupture or extreme thinning of the scar was not recognized, or, if recognized, was not reported.

Naturally, the questions arise: What is the causative factor in the production of the thin, imperfect scar, and what are the conditions predisposing to its rupture? Numerous explanations and theories have been offered which may be roughly classified under the following headings:

1. Infection.
 2. Improper suture technique.
 3. Unsuitable suture material.
 4. Location of the uterine incision.
 5. Involvement of the placental site at operation.
 6. Implantation of the placenta over the scar, with inversion of fetal elements.
 7. Miscellaneous.
- We shall consider each group in some detail.

1. INFECTION

The important rôle played by infection in the production of an imperfect scar is so generally recognized that a case of rupture or extreme thinning is seldom reported without emphasis being laid upon the presence or absence of fever during the preceding puerperium. Losee asserts that "when infection takes place and the cut surfaces are infiltrated with leukocytes and serum, associated with more or less necrosis, then only that muscle tissue remains which has not become necrotic." The extreme necrosis which may take place in an infected wound is well illustrated by Fig. 1. Findley states that failure to secure perfect healing is partly due to septic infection of the wound, and calls attention to the possible existence of a latent gonorrhœal infection, which "may defeat the most painstaking efforts to secure perfect wound healing." The character of the puerperium was noted in 66 of the 97 cases of rupture studied by Holland, who found that 51 were febrile and 15 afebrile. In commenting upon these figures, he asserts that "infection of the uterine wound may occur without rise of temperature, or with only a very slight one; for example, few

will deny that the presence of extensive adhesions to the scar at a subsequent operation is evidence of sepsis during the healing process."

In our opinion the latter part of Holland's statement does not necessarily hold true, and it is permissible to suppose that adhesions may form in the absence of infection, whenever there is a rough, raw surface, such as occurs when the uterine wound has been improperly sutured. For example, in the first case reported in our series the wound had been closed with interrupted silkworm-gut sutures and the stiff knots had been left upon the surface. The puerperium was normal and the scar well-nigh perfect, but nevertheless was covered by dense adhesions. Consequently, if, as is likely, such adhesions resulted from irritation by the stiff knots, it behooves all operators to secure accurate coaptation between the cut edges and to do as little damage as possible to the covering serosa. It must be admitted that imperfect scars may result when the temperature has not been elevated, and in such cases some causative agent other than infection must be sought, while, on the other hand, a scar sufficiently strong to withstand the strain of labor may result even though the record of an elevated temperature indicates that infection had occurred. Here it must be assumed that the infectious process did not involve the uterine incision, or, if it did, that no extensive necrosis resulted.

In the 183 Cæsarean sections, which were done in the service up to the end of December, 1920, there was only one instance of actual rupture of the scar and in this patient the temperature had been elevated for 10 consecutive days following the primary operation. In 15 cases in which the uterus was removed later and studied, and in which we had definite information as to the character of the preceding puerperia, seven were found to be febrile and eight afebrile. In the first group, no trace of the scar could be found in six specimens, while it was only slightly thinned in one. In the second group, the scar could not be located in three instances, while the healing was good in three, fair in one, and poor in one case. Moreover, in three cases the uterus was removed at the third section, so that in such specimens we can only speculate as to which operation had given rise to the scar in question. Of the 13 patients in whom delivery was effected through the natural passages following Cæsarean section, six presented a preceding febrile puerperium.

Such figures, while suggestive, only serve to show how difficult it is to predict the strength or weakness of the Cæsarean scar in the individual case. However, it would seem permissible to infer that the uterine scar will be weak rather than strong if frank infection has occurred in the preceding puerperium, and that such patients should not be subjected to the strain of a prolonged labor, particularly if the original section has been performed on account of pelvic dystocia. On the other hand, when it has been done for some temporary indication, or if there is only moderate pelvic contraction with a small child, even though fever has complicated the former puerperium, we believe that the patient may be allowed to go into subsequent labor, provided that she be kept under careful observation. This view is at variance with that recently expressed by Newell: "It [repeated Cæsarean section] should, however,

be considered obligatory in patients who give a history of a febrile convalescence, since this points to the probability of uterine infection, and it is in these patients that the rupture of the scar in subsequent pregnancies and labors is most to be feared."

2. SUTURE TECHNIQUE

Hurried closure of the uterine incision without due care in accurately approximating the cut surfaces, the placing of the sutures too widely apart, and the inclusion of the decidua in the line of sutures may all be factors in producing an imperfect scar. In this clinic the incision is closed with two layers of catgut, the first consisting of deep buried interrupted sutures placed at intervals of about 1 cm., while a superficial running suture brings together the serosal edges. If, after the first layer of sutures has been placed, there is still considerable gaping of the muscularis, an additional continuous buried suture is used. The decidua should be avoided, since any bits of it inverted into the wound may proliferate and develop into areas of unusual friability. Figs. 2 and 3 illustrate the technique.

Fischer, in 1912, directed attention to the danger of suturing the uterus before it has firmly contracted and retracted, and consequently Green, in 1916, advised that the operation should not be undertaken until labor had been in progress for several hours. "It stands to reason," he says, "that the scar will be thicker and stronger if the closing sutures are applied to a uterine wall thickened by several hours of contractions, than when placed in the thin, comparatively flabby wall of a uterus incised before labor has begun." Apparently the rationale of this theory rests upon a mechanical basis; for, if the sutures be laid and tightly tied while the musculature is lax, it is conceivable that when the uterine wall increases in thickness as the result of subsequent contraction, the sutures will either become untied, or else they will tear through the thickened tissue. In the latter event a gap may be left on the inner surface of the uterus, which is invaded by endometrium. Unfortunately, however, it is not always possible to wait for the occurrence of firm contraction, as brisk hemorrhage may necessitate immediate suturing of the wound. Figs. 4 and 5 illustrate the assumed sequence of events.

Analysis of the operative notes concerning the first operation in our patients, from whom the uterus was removed at a subsequent section and its scar studied microscopically, shows that the character of the uterine contraction was recorded in 11 instances. In eight, firm contraction had occurred before the sutures were placed, and in five of these the scar was normal or could not be found, while in the three others definite thinning was evident. In one of the latter the convalescence was complicated by an outspoken infection, so that it is difficult to determine whether the infection or the slow muscular contraction had played the more important part. On the other hand, in the three instances of poor contraction, there was no trace of the scar in two, while it was very thin in the third case. Moreover, in the one instance in which the old scar ruptured in a subsequent pregnancy, it had been necessary to suture the uterus while its musculature was lax.

It must be admitted that the figures available are too few to permit final conclusions, but they clearly indicate that suture of the uterus after firm contraction has occurred does not necessarily insure an ideal scar.

3. SUTURE MATERIAL

The fact that a wide variety of suture material has been used in closing the uterine incision is sufficient proof that none is entirely satisfactory from every point of view. Plain, formic, and chromicized catgut, silkworm-gut, kangaroo tendon, ordinary black or white silk, metallic wire, etc., have been employed at one time or another. Plain catgut has generally been discarded on account of its rapid absorptibility, although Carstens advocates it for that very reason.

In our hands chromic catgut has given relatively satisfactory results. Holland, on the contrary, believes that it is also absorbed more rapidly than is desirable, particularly in the presence of infection. Upon the analysis of a large number of cases, he found that subsequent rupture occurred two and a half times more frequently after the use of catgut than of silk. Consequently, he is of the opinion that non-absorbable sutures are preferable, and holds that silkworm-gut fulfills the requirements most satisfactorily. Plain silk is a notorious harbinger of bacteria, as is admirably illustrated by Case X below. Prusmann objects to non-absorbable sutures on the ground that small channels may be formed about them, and as these may be invaded by bacteria or even by endometrium, necrosis or weakening of the scar may result. Eckstein, in 1904, indicated the advantages of metallic sutures, and suggested the use of thin flat bands of lead, which, in addition to being non-absorbable, would serve as supports for the scar in future pregnancies.

In our series of operations silkworm-gut was used only in a single instance, and the resulting scar was ideal. When the uterus was removed a year later, the sutures were still *in situ* (Fig. 6), but, as has been mentioned previously, dense adhesions had developed along the entire length of the scar, probably as the result of the knots irritating the surrounding tissues. It would seem, therefore, that when this material is used the sutures should be buried, and the superficial muscle layer and peritoneum brought together with catgut over it.

Obviously, the choice of suture material is at present a matter of personal preference, and no definite statement can be made as to which possesses the greatest merit. To my mind, the technique used in laying the sutures is of greater importance than the material of which they are composed.

4. SITUATION OF THE INCISION

At a Cæsarean section the uterus may be incised in any one of the following four locations; (1) in the midline of the anterior wall; (2) in the posterior wall; (3) in the fundus, either transversely or longitudinally; and (4) in the lower uterine segment, likewise either longitudinally or transversely. In the last group may be included those cases of vaginal hysterotomy, in which the lower uterine segment becomes involved in the incision. Without doubt, in the great majority

of cases the median anterior wall is the site of election, although in recent years there has been a growing tendency to advocate incision of the lower segment. The uterus is opened through the posterior wall only in the rare instances in which its body has undergone extreme forward displacement.

Varying results have been obtained with the several modes of incision and a voluminous literature has accumulated upon the subject. In 1897, Fritsch described his transverse fundal incision, which has been discarded after a number of years of experimentation. Eckstein, in 1904, reported the first case of rupture of a fundal scar in a subsequent pregnancy, and following it numerous similar reports have appeared in the literature. Scheffzek called attention to the fact that in order to secure perfect healing of any wound, its edges should be immobilized, and admitted the impossibility of keeping the actively contracting and relaxing uterus entirely at rest. Furthermore, he pointed out that the fundal incision approached the ideal even less than one through the median wall or the lower uterine segment. In proof of this he reported the findings at autopsy upon a woman who had died from a toxæmia several days after a Cæsarean section, which showed that the sutures in the fundal wound had become loosened or entirely untied, presumably as the result of active uterine contractions. By way of contrast, he also reported his observations upon 10 women who had been subjected to extraperitoneal Cæsarean section. In five of them repeated section was necessary; while of the other five, one was delivered spontaneously, and in four labor was induced in the latter part of pregnancy. In each instance the uterine scar remained intact, although in one the pains continued for 68 hours before delivery was effected. Consequently, he concluded that the scar is much more resistant after extraperitoneal section than after either the fundal or median incision. Spalding holds that "the extraperitoneal Cæsarean section carries with it a better prognosis than the classical abdominal operation." Furthermore, Holland concluded that transverse fundal scars are especially liable to rupture, and Offerman, in 1916, collected 21 such accidents from the literature. In addition to this unusual liability to rupture, he feels that adhesions are especially prone to occur, and that in those cases in which infection of the uterine wound occurs, drainage will be into the abdominal cavity rather than through the abdominal wound, thereby materially decreasing the chances for recovery.

Fischer has described in detail two uteri which had been subjected to previous fundal incisions. In one a complete rupture of the scar occurred in a subsequent pregnancy, while in the other it was so thin that rupture would have undoubtedly occurred had labor not been terminated by operation. From this experience he is convinced that the fundal incision should not be used. Findley has gone even further, and says that "the transverse fundal, extraperitoneal, and cervical incisions have not lessened the liability of rupture in subsequent labors, but on the other hand have probably increased the hazard."

Since our experience with the extraperitoneal and cervical incisions has been limited to four cases, we do not feel quali-

fied to make definite statements concerning their comparative merits. In none of our cases did rupture occur, although in one instance the woman subsequently went through a moderately severe spontaneous labor. Moreover, in two of the uteri which were removed at a subsequent section, examination revealed no trace of the old scar. Rohrbach claims that the scar following the cervical operation practically insures against danger of rupture in future pregnancies, and it is undoubtedly true that only a few instances of this accident have been reported. It must, however, be admitted that this is too favorable a verdict, as Wolff, Franz, Freund and Labhardt have each described cases of rupture. Baisch, in 1920, reviewed the results following 170 Cæsarean sections in the Stuttgart clinic, and attributed the good results to the employment of the transperitoneal cervical operation. Twenty-four of his patients had repeated sections, and in three rupture of the uterus occurred. In the first case it was unquestionably through the old cervical scar; while in the other two he attempts to prove that it was not. In the second case, although the rupture involved the scar, he argues that it had originated in the body of the uterus, as at the first operation the incision had torn upward during extraction of the child so that only a part of it could be covered with the bladder. In the third case the patient had a bicornate uterus in which three pregnancies had occurred. The first was terminated by a transverse fundal incision, the second by a cervical incision, while the third terminated by rupture. On opening the abdominal cavity at that time he found that the cicatrix of the first operation had yielded, while the one in the lower segment was intact. He considers this an excellent demonstration of the superiority of the cervical operation. We, however, cannot agree with his contention; for, even accepting his argument concerning the second case, which appears questionable, he admits one cervical rupture in 24 repeated sections, which is somewhat higher than the 3 per cent incidence following the usual incision.

Before concluding the consideration of the location of the uterine incision, I shall refer to two cases of repeated Cæsarean section which possess points of unusual interest, one reported by Planchu, the other by Harrar. In the former, the three linear scars were plainly visible in the anterior median line at the time the fourth consecutive section was performed. They were equidistant and approximately at the same level and were included in a broad band of thinning—2.5 cm. wide, where the muscle had been replaced in large part by fibrous tissue. An explanation of the mode of production of such a condition is afforded by Harrar's case. Here the uterus had ruptured after a third Cæsarean section, and upon examining the specimen he found that the line of rupture did not involve an old incision but had occurred between two of them. As the distance between the scars was one centimeter, he inferred that a danger zone had been created at the previous operations by cutting the trophic nerves and thereby interfering with the blood supply in that neighborhood. To obviate such a danger he urges that in a repeated Cæsarean section the inci-

sion should be made some distance away from the old scar, or else that the latter should be completely resected.

5. INCISION INTO THE PLACENTAL SITE

Writers upon the subject have advanced two reasons for assuming when the uterine incision involves the placental site that the resulting scar will, in some cases, be thinner than it would have been had the incision been elsewhere. First, as the thickness of the uterine wall at the placental site is several millimeters less than elsewhere, it is argued that the scar will be thinned in like proportion. Second, the foetal elements may interfere in the healing process. The latter view is held, in a general way, by Spalding, Fischer, and others. However, they, and practically all observers, believe that this is only an accessory factor of no great importance.

For the purpose of determining what relation, if any, incision through the placental site has upon the healing of the uterine wound, I have divided our cases into two groups. The first includes those in which the location of the placenta was noted at the first operation and in which the uterus was removed at a subsequent section and studied histologically; while the second includes patients who were delivered by the natural passages following a preceding section in our clinic. The former group includes 17 specimens, and the latter six cases. In the first group the incision involved the placental site in seven instances, and of these, two uteri showed no trace of the scar, in two there was very slight thinning, while in the remaining three the thinning was quite marked. In the 10 instances in which the placental site was not involved, no trace of the scar was visible in three, it was satisfactory in six, and was poor in one uterus. In the second group the placental site had been incised in two patients, and not in the other four. It may be added that in the single instance of rupture occurring in our series and reported below, the operative note concerning the first section failed to mention the location of the placenta.

From these figures the hasty observer might be led to the conclusion that a definite relation exists between imperfect healing of the scar and the involvement of the placental site at operation. Our observations do not prove it, and in addition it should be remembered that such few figures as are available do not justify binding conclusions. Consequently, we can only say that our findings merely point to the necessity of closer observation of the influence of this factor in the future.

6. IMPLANTATION OF THE PLACENTA OVER THE OLD SCAR

Eckstein, in reporting his case of ruptured Cæsarean scar, laid particular emphasis upon the insertion of the placenta over the old scar as a probable cause of the rupture. He assumed that the foetal elements invaded and weakened the scar, much in the same way as occurs in tubal pregnancy. Spalding states that "while it is plausible that the placental elements might lead to poor healing of the wound in cases where the incision is into the placental site, there is hardly

sufficient evidence available to uphold the idea that the syncytium will attack sound scar tissue any more than it will attack sound uterine tissue." Consequently, he believes that the placenta, when inserted over the scar, may even act as a splint and serve to support the weakened uterine wall. At the same time he calls attention to the possibility that the formation of small retroplacental hæmatomata may eventually cause the rupture of the scar as they grow larger. In 1906, Couvelaire reported a series of nine cases of ruptured Cæsarean scar and stated that in five of the six instances in which the placental attachment had been noted, implantation had occurred over the old scar. He therefore concluded that the insertion of the placenta over the scar cannot be considered the sole cause of rupture, but that when it is already thin its invasion by foetal elements must lead to further thinning and accentuation of its friability.

Our observations concerning the bearing of this factor indicate that it is not important. Strange to say, in each of the three uteri which had been removed after two previous sections the placenta at the third operation was implanted over the old scar. In the first case, both scars were seen and were quite thin; in the second, only one scar was visible and it, likewise, was considerably thinned; while in the third, neither scar was visible. In 17 uteri which had been removed at the second operation, the placenta was implanted over the scar in three. In two of these the scar of the previous section could not be found, while it was well healed in the third uterus. Furthermore, in the one case of rupture the placenta was inserted over the old scar, but in none of these cases was there any evidence that the scar had been invaded by foetal elements.

7. MISCELLANEOUS

(a) *Excessive Distention of the Uterus*.—In the cases of rupture reported by Woyer, Couvelaire, and Scheffzek, the presence of hydramnios or multiple pregnancy had subjected the uterus to abnormal distention, which was assumed by these authors to have been a predisposing factor. On the other hand, Gilles, in 1916, reported the successful termination of a twin pregnancy without rupture of the uterus, in spite of two previous Cæsarean sections. The combined weight of the two children was 4950 grams.

(b) *Forcible Intrauterine Manipulations*.—Version and extraction, the use of hydrostatic bags, uterine tampons, etc., have been mentioned as possible causes of rupture, although it is only in cases of marked thinning of the scar that such factors are of any importance.

(c) *"Water-Wedge Theory"*.—Spalding, in attempting to explain the rupture of the scar in a certain percentage of cases, advanced the theory that a small water-wedge formed by the amniotic sac may serve to dilate the thinned-out scar in exactly the same way that the bag of waters brings about dilatation of the cervix in normal labor.

Before passing on to the consideration of the anatomy of the Cæsarean scar, we shall refer to several investigations concerning its resistance, and then say a few words concerning the incidence of adhesions and their mode of production. In 1910,

Mason and Williams studied the resistance of the Cæsarean scar experimentally. They subjected a number of pregnant animals to Cæsarean section and some time later removed the uterus for investigation. They attached weights to a strip of muscle containing the scar and increased them until rupture occurred, when it was found that it took place through the sound muscle and not through the scar itself. Their experiments gave such uniform results that they drew the conclusion "that a firmly united scar is even stronger than the intact uterine muscle and should easily be able to withstand any strain which the latter is capable of bearing." Moreover, the case reported by Harrar demonstrated that the normal muscle tissue may yield before the cicatrix, although the myometrium in that instance was regarded as having become "devitalized." As anatomical study of the well healed scar shows no reason why it should rupture before the surrounding muscle, it is probable that the statement of Mason and Williams holds true for a "firmly united scar." In the thin, poorly healed wound, on the other hand, there can be little doubt that the rupture occurs directly through the scar and not through the adjacent tissue.

ADHESIONS

That adhesions are quite common following Cæsarean section is clearly demonstrated by the following observations made in our clinic at repeated operations.

TABLE I

	Adhesions			
	Dense	Filmy	Moderate	No note
After one previous section.....	17	7	1	4
After two previous sections.....	6	1	0	0

TABLE II

	Puerperium	
	Febrile	Afebrile
Dense adhesions.....	8	7
Filmy adhesions.....	5	3
Moderate adhesions.....	1	1
No note.....	2	1

Table II illustrates the fact that while adhesions are more commonly formed after a febrile puerperium, yet the absence of fever does not necessarily mean that adhesions have not formed.

It is difficult to determine just how much harm such adhesions do. Doubtless, they may give rise to considerable abdominal discomfort, which may occasionally be so great as to necessitate a second operation; and Humpstone reports an instance in which it was necessary to remove the uterus

before the symptoms disappeared. In other patients the adhesions may be so dense that the subsequent operation is performed under tremendous difficulties, and in rare instances regrettable accidents, such as injury to the gut or bladder, can be directly traced to their presence. For example, in Case XIX described below, after the adhesions had been freed, the anterior surface of the uterus presented such an extensive raw bleeding area that hysterectomy was necessary.

Can the formation of adhesions be prevented? Davis has suggested that the liability toward their development may be lessened by resorting to the high abdominal incision. This, however, is a mere supposition, and inasmuch as the advantages of the lower incision are so great, we do not feel that it should be discarded in favor of the higher one. Consequently, it seems that the only way at present by which we can lessen the incidence of adhesions in the absence of infection is by securing as perfect coaptation between the cut surfaces as possible, thereby leaving a minimal area which can excite mechanical irritation.

ANATOMY OF THE CÆSAREAN SECTION SCAR

For convenience in description Cæsarean scars may be divided into two main groups: first, those which have ruptured or were on the point of rupturing when the abdomen was opened; second, those in which satisfactory union has persisted.

As Losee and also Couvelaire have pointed out, the first step in the mechanical healing of the uterine scar is the deposition of fibrin between the edges of the wound, which acts as a framework for the ingrowing tissue which will eventually determine the character of the cicatrix. In spite of the constant contraction and relaxation of the uterus, if infection is absent, and the sutures have been so laid that the edges of the incision remain approximated, firm union will almost invariably result. On the other hand, if there is necrosis due to infection, or if the sutures tear through, leaving a gaping wound, there is an ingrowth of endometrium, which may involve almost the entire thickness of the wound. Such a scar, when seen in a subsequent pregnancy, is made up almost entirely of decidua and peritoneum, and its gross appearance in the unopened distended uterus is characteristic. Instead of the vertical shallow depression seen in the more perfectly healed scars, it is represented by a glistening translucent band of tissue which bulges outward beyond the general surface; while after the uterus has been emptied, hardened, and cut in cross-section, the scar is marked by very deep depressions on both the outer and inner surfaces of the uterus. On microscopical examination it will be found that it is composed almost entirely of thickened and highly vascularized serosa overlying the decidua. There is an increase in the fibrous tissue and a definite decrease in the elastic fibres, while occasionally a few muscle fibres may be present.

Between a scar of this nature and one which has healed perfectly all gradations may be observed in so far as gross appearance and thickness are concerned. Nor is the microscopical picture markedly different in the thin scar as con-

trasted with the well healed one, except when there has been no muscular union. Figs. 7, 8 and 10 depict the characteristic appearance of well healed scars, while Fig. 9 (A) shows a scar which is considerably thinner than the other three. In all there is a slight depression both upon the inner and outer surfaces of the uterus, while the cicatrix itself appears as a white line joining the two depressions. Unless such scars are examined under the microscope, one is apt to infer that they are made up of fibrous tissue alone. Such, however, is not the case, and in all four specimens muscle fibres can be seen running directly across the old line of incision, with no break in their continuity and with only slight distortion of their course.

Jolly and others have reported instances of partial rupture of the old Cæsarean scar. Upon studying such specimens in detail, they found that one portion of the scar was composed solely of decidua and peritoneum, while the rest was made up of normal muscle. Similar variations in thickness throughout the length of the same scar are well illustrated by Figs. 15 and 16, which are photographs from the two sides of a block one centimeter thick, and show that within so short a distance the thickness of the scar may be doubled.

Briefly then, Cæsarean section scars vary considerably in thickness, depending upon the accuracy with which the margins of the wound have been brought into apposition. Under ideal conditions the muscle unites perfectly, and its fibres cross the site of the incision as if it had never been made. When, however, there has been no muscle union, the scar is made up solely of decidua and peritoneum. Most scars are marked by funnel-shaped depressions on the outer and inner surfaces of the uterus. The muscle bundles are not much distorted, the formation of fibrous tissue is much less than would be anticipated, and when it is increased, the elastic fibres are correspondingly diminished in number. (See Fig. 7.)

Fig. 7, which represents a low power microscopic picture, gives an idea of the characteristic conditions obtaining in a well healed wound, while the photographs accompanying some of the case reports clearly depict some of the gross variations which may be encountered.

In the case reports which immediately follow, we shall describe the gross appearance and microscopic structure of the Cæsarean scar as it appeared in 21 uteri which were removed at a second or third section, and which will serve to amplify and reinforce what has already been said.

CASE REPORTS AND DESCRIPTION OF SPECIMENS

In the following cases the uterus was either removed at operation or secured at autopsy. A brief clinical synopsis precedes the more detailed description of the specimen.

CASE I.—House No. 1274, 24 years, black. Generally contracted rachitic pelvis, D. C. 10.5 cm.

Three vaginal deliveries. Fourth labor ended by Cæsarean section after a second stage of three hours. Child dead—4180 gm. Incision through the placental site; closure with interrupted silk-worm-gut sutures. Puerperium normal except for a rise of temperature to 100.4° on the second day. Fifth labor ended by Cæsarean section followed by supravaginal amputation of the uterus. Uneventful recovery.

Omental adhesions, placenta on posterior wall. No note as to the condition of the cicatrix before the incision of the uterus.

Description of Specimen.—After hardening, the uterus measures 14 x 12 x 10 cm. On the midline of its anterior surface is a mass of adhesions, evidently over the scar of the previous operation. On removing them, a linear depression 8 cm. long becomes visible, with the silk-worm-gut sutures still in place. (Fig. 6.) On cross-section through the uterus the scar is represented by depressions on its inner and outer surfaces, which are joined by an irregular whitish line (Fig. 8). The uterine wall is 11 mm. thick at the site of the scar, as compared with 17 mm. adjacent to it, a ratio of 1 to 1.54. On microscopical examination no trace of the scar is seen except for the funnel-shaped depressions and a slight irregularity in the arrangement of the muscle fibers. The external depression is filled with highly vascularized connective tissue, while the inner one is lined with decidua containing many glands. In the center of the scar is a small island of characteristic decidual tissue. The Weigert stain shows a decrease in the elastic tissue at the site of the scar.

CASE II.—House No. 7145, 27 years, black. Generally contracted rachitic pelvis, D. C. 8.75 cm.

First labor, spontaneous, premature; second, pubiotomy. The third and fourth pregnancies were ended by Cæsarean section with silk sutures. Ideal recovery after both operations except for a mastitis in the fourth puerperium. The placenta was on the posterior wall in the first, and on the anterior wall in the second section. The fifth pregnancy ended in a 3 months' abortion, and the sixth was terminated by a Porro section. Puerperium normal except for a mastitis. There were dense adhesions between the uterus and anterior abdominal wall, but no traces of the cicatrices of the previous Cæsarean sections were visible. The placenta lay anteriorly.

Description of Specimen.—After hardening, the uterus measures 10 x 11 x 8 cm. Numerous dense adhesions are present over its anterior surface. On either side of the present incision is a vertical depression apparently corresponding to the scars of the previous operations. On cross-section, the scars are clearly visible and are represented by the typical funnel-shaped depressions on the outer and inner walls (Fig. 9). The scar on the left is 6 mm. thick, while the adjacent uterine wall is 23 mm., a ratio of 1 to 4. On the right side the scar is 11 mm. thick, a ratio of 1 to 2. On microscopical examination, except for the thinning of the uterine wall, there is little to indicate the location of the former incisions. There is no scar tissue and the muscle fibers run directly across from side to side. The inner depressions are lined with decidua, while the outer ones are filled with thickened scar-like peritoneum. The elastic fibers are decreased. It is impossible to say which scar belongs to the first and which to the second operation. One is almost twice as thick as the other, and the only factor noted at either operation that might have influenced the healing was the poor contraction of the uterus at the first section.

CASE III.—House No. 6540, 22 years, black. Generally contracted rachitic pelvis, D. C. 9.5 cm.

The first pregnancy ended by a conservative Cæsarean section. The placenta lay posteriorly, the uterus contracted firmly, and the incision was closed with catgut. The puerperium was febrile for five days. The second labor was spontaneous premature at seven months. The third pregnancy was terminated by a Porro section, the operation being performed 19 hours after the onset of labor. There were several dense adhesions between the uterus and the anterior abdominal wall; the placenta was inserted posteriorly; the puerperium was normal. The scar of the previous section was not noted at the time of the operation.

Description of Specimen.—After hardening, the uterus measures 17 x 12 x 9 cm. The posterior surface is free except for a few velamentous adhesions. On its anterior surface the scar of the previous operation is indicated by a deep depression, from the lower angle of which a broad adhesion extends down to the free peritoneal margin. On cross-section (Fig. 10), the scar is represented by funnel-shaped

depressions on both the outer and inner surfaces of the uterus. Joining them is a white puckered line resembling scar tissue. The uterine wall measures 28 mm. in thickness, while the scar is 22 mm. thick, a ratio of 1 to 1.36. On microscopical examination the only trace of scar consists in a slight irregularity in the arrangement of the muscle fibers and some increase in the fibrous tissue. The elastic tissue is decreased.

CASE IV.—House No. 6847, 24 years, black. Generally contracted rachitic pelvic, D. C. 9.25 cm.

First pregnancy ended by a conservative Cæsarean section. The placental site was incised, the uterus contracted firmly, and closure was with catgut. Puerperium uncomplicated.

Second pregnancy ended by a Porro section. Two broad adhesions attached the anterior surface of the uterus to the abdominal wall. The placenta lay posteriorly. No sign of the old uterine scar. Puerperium normal.

Description of Specimen.—After hardening, the uterus measures 19 x 13 x 10 cm. Two broad bands of adhesions on the anterior surface. Careful examination, grossly and microscopically, shows no trace of the old scar.

CASE V.—House No. 6939, 18 years, black. Generally contracted rachitic pelvis, D. C. 10.25 cm.

First pregnancy ended by a conservative Cæsarean section. Placental site not incised; closure with catgut. The character of the uterine contraction not recorded. The puerperium was complicated by wound infection, the temperature being elevated for 15 days. The second pregnancy was terminated by a Porro Cæsarean section. In attempts to extract the child through too small an incision, the uterus was torn down to the bladder and into the right broad ligament, necessitating its removal. The placenta was inserted posteriorly. The puerperium was normal except for a simple mastitis.

Description of Specimen.—After hardening, the uterus measures 15 x 12 x 10 cm. The surfaces are free from adhesions and there is no trace of the old scar.

CASE VI.—House No. 6076, 23 years, white. Generally contracted rachitic pelvis, D. C. 10 cm.

The history of the first two labors is meagre. Both were instrumental and both children were stillborn. The third labor was terminated by a conservative Cæsarean section. The placental site was not involved, the uterine contraction was poor and closure was with catgut. Puerperium febrile for six days. Fourth labor ended by a Porro section. There were a few filmy adhesions on the anterior surface of the uterus. The placenta lay posteriorly. Puerperium normal.

Description of Specimen.—After hardening, the uterus measures 14 x 14 x 8 cm. A few thin adhesions cover its anterior surface, in the midline of which is a smooth shallow depression indicating the site of the previous operation. On cross-section, except for this depression and a similar one on the inner surface, there is no trace of the scar. The uterine wall measures 26 mm. in thickness, the scar 23 mm., a ratio of 1 to 1.13. Microscopically there is no sign of scar tissue, and the muscle bundles show complete regeneration.

CASE VII.—House No. 8087, 29 years, black. Generally contracted rachitic pelvis, D. C. 8.5 cm.

The first and second labors were ended by a conservative Cæsarean section and a destructive operation, respectively, both operations being performed elsewhere. The third pregnancy was terminated by a Porro section. Several loops of gut were densely adherent to the anterior abdominal wall and anterior surface of the uterus. The placenta was inserted posteriorly. Puerperium normal.

Description of Specimen.—After hardening, the uterus measures 15 x 16 x 7 cm. On the anterior surface is a depressed raw area, representing that portion which was covered by adhesions, the latter overlying the scar of the previous operation. On cross-section, the scar is represented by shallow depressions on the outer and inner surfaces of

the uterus. The thickness of the scar is 19 mm., that of the adjacent wall, 25 mm., a ratio of 1 to 1.3. Microscopically, the inner depression is seen to be filled with decidua, while the outer is lined with vascular serosa. The muscle fibers run directly across the line of the former incision without break in continuity. There is no increase in fibrous tissue.

CASE VIII.—House No. 8137, 31 years, black. Generally contracted rachitic pelvis, D. C. 9.5 cm.

The first pregnancy was terminated by a conservative Cæsarean section. The placental site was incised, the uterus contracted firmly; closure with catgut. Puerperium normal. Second labor ended by a destructive operation elsewhere. Third labor ended by a Porro section. There were a few thin omental adhesions on the anterior surface of the uterus, but no sign of the previous scar. Placenta inserted posteriorly. Puerperium normal.

Description of Specimen.—After hardening, the uterus measures 16 x 14 x 6.5 cm. Upon the anterior wall, half a centimeter lateral from the present incision is a slight vertical depression, 2 mm. deep, and 4 cm. in length. On cross-section, the usual funnel-shaped depressions are seen on the outer and inner surfaces. The scar measures 18 mm. in thickness, the adjacent uterine wall, 22 mm., a ratio of 1 to 1.2. Microscopically, no trace of the scar can be found except for the depressions just mentioned. There is no increase in fibrous tissue.

CASE IX.—House No. 8332, 21 years, black. Generally contracted rachitic pelvis, D. C. 9.5 cm.

The first pregnancy was terminated by a conservative Cæsarean section. The site of the placental attachment was not recorded; uterine contraction poor; closure with catgut. Puerperium normal. A conservative Cæsarean section ended the second pregnancy. There were numerous filmy adhesions near the fundus, while lower down was a single broad adhesion running to the old abdominal scar. The placental site was incised, the uterus contracted firmly, and closure was with catgut. The scar of the previous operation was not visible. The puerperium was complicated by infection. The abdominal wound broke down on the sixth day, and a sinus developed which extended well down into the uterus. A pelvic abscess was opened and drained through the vagina on the seventeenth day. The third pregnancy was terminated by a Porro section. Dense adhesions covered the entire anterior surface of the uterus. The placenta lay anteriorly. Puerperium normal.

Description of Specimen.—After hardening, the uterus measures 15 x 13 x 6 cm. The present incision lies to the left of the midline. The entire anterior surface presents a ragged appearance due to adhesions. In the midline the scar of one of the previous operations is marked by a slight depression. On cross-section there is a similar, though much deeper, depression on the inner surface, but there is no trace of a second scar. The scar is 20 mm. in thickness; of this, 4 mm. is made up of fibrous peritoneal tissue, 4 mm. of muscle, and 12 mm. of a triangular wedge of decidua. The adjacent uterine wall measures 22 mm. On microscopical examination it is seen that, although there has been some muscle reunion, the individual bundles are greatly distorted, with a marked increase in the fibrous tissue in its locality. Presumably this scar is the result of the second operation.

CASE X.—House No. 8679, 24 years, black. Generally contracted rachitic pelvis, D. C. 10.25 cm.

The first pregnancy was terminated by a conservative Cæsarean section elsewhere. Three years later the patient was admitted to the surgical service of this hospital with the following history: Fifteen months after the Cæsarean section the abdominal wound began to drain, the discharge consisting mainly of blood, but occasionally containing pus. A sinus tract, which extended down into the uterine cavity, was dissected out, and a number of heavy black silk sutures were removed from the uterine wall. Recovery was uneventful. Sixteen months later the second pregnancy was terminated by a Porro section. A few thin omental adhesions were attached to the anterior

surface of the uterus. Placenta posterior. Puerperium normal except for a simple mastitis.

Description of Specimen.—After hardening, the uterus measures $13 \times 10 \times 7$ cm. To the left of the present incision is a slightly depressed area 2 mm. wide, and 7 cm. long, which apparently represents the old scar. On cross-section, however, there is no depression on the inner surface of the uterus, and microscopically no trace of the scar can be found.

CASE XI.—House No. 8826, 24 years, black. Generally contracted rhachitic pelvis, D. C. 9.75 cm.

The first labor ended spontaneously after 30 hours, the child being stillborn. The second pregnancy was terminated by a conservative Cæsarean section. The placental site was incised, the character of the uterine contraction was not recorded; closure with catgut. Temperature elevated to 101.4° on the second day. The third pregnancy was also ended by a conservative Cæsarean section. Several loops of small intestine were adherent to the abdominal scar and one of them was accidentally opened. It was repaired at once with silk. The omentum was adherent to the fundus. The placental site was incised, the character of the uterine contraction was not recorded; closure with catgut. Puerperium normal. The fourth labor was terminated by a third Cæsarean section and sterilization effected by resection of the tubes. Again an adherent loop of gut was opened, and was repaired with silk. A few omental adhesions covered the fundus. The placental site was incised, the uterus contracted well; closure with catgut.

On the third day of the puerperium the abdominal wound broke down, exposing the anterior surface of the uterus. This was covered with small glistening blebs filled with gas. A drain was inserted and the wound closed with silkworm-gut. Cultures of the wound showed *B. aerogenes capsulatus*, *B. coli*, *Staphylococcus aureus*, and *streptococci*. The patient died 12 hours later and autopsy showed a generalized peritonitis (gas bacillus); hemorrhage from the uterine incision; diphtheritic colitis; epithelial necrosis of the kidneys.

Description of Specimen.—After hardening, the uterus measures $17 \times 12 \times 6$ cm. On the anterior surface is a depressed area, more or less covered by fine adhesions, corresponding to the partially broken down wound of the recent operation. Several of the catgut sutures have become untied, and for a distance of 2 cm. at the upper angle of the incision there has been complete separation of its edges. On cross-section (Fig. 11), it is seen that the cut surfaces are covered by necrotic tissue and at no place is there any sign of firm union. The scars of the previous operations are not visible. Microscopical examination shows that the decidua has been replaced, in large part, by fibrin, leucocytes, and necrotic tissue. The musculature presents a peculiar areolated appearance due to the formation of gas pockets. In many places the individual muscle cells are undergoing degeneration. There is no evidence of beginning muscular union.

CASE XII.—House No. 9115, 19 years, black. Pelvis normal.

The first pregnancy was terminated elsewhere by a Cæsarean section, the indication being "convulsions." The patient was admitted to this hospital in the eighth month of her second pregnancy. Blood pressure 180; the urine contained 3 gm. of albumin per liter. Labor was induced by means of a bougie. After pains had lasted for 12 hours, the patient had her first convulsion, followed in a few minutes by a second. As the cervix was still undilated and the child dead, it was decided to remove the uterus unopened. The omentum was adherent to the anterior surface of the uterus. Puerperium febrile.

Description of Specimen.—After hardening, the uterus measures $22 \times 16 \times 13$ cm. On the anterior surface are a few thin adhesions. No trace of the old scar. On cross-section the placenta is found to lie anteriorly. There is no sign of the usual depressions which so frequently represent the line of former incision. Microscopical examination shows no increase in fibrous tissue. The Weigert stain shows the presence of large numbers of streptococci in the decidua, thus

demonstrating the wisdom of removing the uterus, rather than being content with a conservative operation.

CASE XIII.—House No. 9323, 33 years, black. Generally contracted rhachitic pelvis, D. C. 9 cm.

The first pregnancy ended in a spontaneous abortion at the third month, the second in a conservative Cæsarean section. The placenta lay posteriorly; character of the uterine contractions not recorded; closure with catgut. The puerperium was febrile for the first three days. The third pregnancy was terminated by a Porro section, the vulva being so completely covered by a foul, sloughing mass of condylomata that a conservative operation would have almost certainly been attended by infection. There were no adhesions, and the placenta lay beneath the old scar. Puerperium normal.

Description of Specimen.—After hardening, the uterus measures $15 \times 11 \times 6$ cm. Extending from the fundus downward for a distance of 10 cm., and with its lower half slightly to the right of the present incision, is a vertical depression, representing the scar of the previous operation. It averages 1.5 cm. in width, and its deepest portion lies 0.5 cm. beneath the general surface (Fig. 11). It presents a number of transverse depressions, which are apparently due to the individual sutures. On cross-section (Fig. 12) the scar is marked by typical depressions on both surfaces, whose tips are 16 mm. apart, as compared with 25 mm. in the adjacent uterine wall, a ratio of 1 to 1.59. On microscopical examination there is considerable distortion of the individual muscle bundles, although they definitely run from side to side without break. There is no increase in the fibrous tissue.

CASE XIV.—House No. 9539, 24 years, black. Generally contracted rhachitic pelvis, D. C. 8 cm.

First pregnancy terminated by conservative Cæsarean section elsewhere. She was admitted in her second pregnancy, after having been in labor for 30 hours with numerous vaginal examinations. A Porro section was done with difficulty on account of dense adhesions between the lower angle of the abdominal scar and the anterior surface of the uterus. The scar of the previous Cæsarean was not visible before the uterus was opened. The placenta lay posteriorly. Puerperium febrile.

Description of Specimen.—After hardening, the uterus measures $15 \times 12 \times 8$ cm. Externally the scar is represented by a very slight depression along the upper third of the anterior wall. On cross-section (Fig. 13) the uterine cavity presents a triangular appearance, the base being formed by the posterior wall and the apex by the funnel-shaped depression on the inner surface of the anterior wall. Upon microscopical examination no trace of scar tissue can be found and the muscle fibers at the site of the scar are only very slightly disarranged.

CASE XV.—House No. 9530, 31 years, white. Simple flat pelvis, D. C. 10.75 cm.

In the first pregnancy the child was delivered by pubiotomy after a second stage of 14 hours, the D. C. at that time measuring 9.75 cm. The second pregnancy was terminated by a conservative Cæsarean section with an uneventful recovery. Incision into the placental site; character of uterine contraction not recorded; closure with catgut. The third pregnancy was ended by supravaginal amputation of the uterus. There were several large adhesions extending from the old abdominal scar to the anterior surface of the uterus. Placenta posterior; puerperium normal.

Description of Specimen.—After hardening, the uterus measures $17 \times 13 \times 7$ cm. There was no trace of the old Cæsarean scar either on gross or on microscopical examination.

CASE XVI.—House No. 10243, 21 years, black. Flat rhachitic pelvis, D. C. 10 cm.

First pregnancy terminated by a conservative Cæsarean section. The placenta lay posteriorly; character of the uterine contractions not recorded; closure with catgut. Puerperium febrile for two days. In her second pregnancy the patient was admitted to the hospital after having been in labor 15 hours with numerous vaginal examinations. Cæsarean section with supravaginal amputation of the uterus, followed

by a febrile puerperium for five days. The placenta was posterior. There was a single broad adhesion attaching the omentum to the anterior surface of the uterus.

Description of Specimen.—After hardening, the uterus measures $14 \times 11 \times 7$ cm. In its contracted condition a number of transverse markings are visible on the anterior surface, apparently corresponding with the sutures of the former operation. On cross-section there is no trace of the scar, except immediately adjoining the uterine cavity where there is an infolding, 7 cm. in length, perhaps indicating that union had been faulty in that location. Microscopical examination shows no trace of scar tissue.

CASE XVII.—House No. 10792, 28 years, black. Generally contracted rachitic pelvis, D. C. 9 cm.

The first two pregnancies were terminated by Cæsarean sections, the first being performed elsewhere. At the second the uterus was found loosely adherent to the anterior abdominal wall. The placenta was inserted on the anterior wall; the uterus contracted slowly, and closure was with catgut. Puerperium febrile for seven days. The third pregnancy was ended by a Porro section. Broad adhesions extended from the abdominal wall to the anterior surface of the uterus. Placenta posterior; puerperium normal.

Description of Specimen.—After hardening, the uterus measures $16 \times 11 \times 5.5$ cm. The entire anterior surface presents a raw surface which was covered by adhesions at the time of operation. A single scar is visible as a slight linear depression not more than 1 mm. in depth on the external surface, while on the inner surface there is a deeper depression, 7 cm. in length (Fig. 14). Cross-sections made at various levels reveal considerable variation in the thickness of the scar (Figs. 15 and 16). On microscopical examination there is a slight increase in the fibrous tissue, but the musculature has regenerated completely and there is no distortion of the individual fibers.

CASE XVIII.—House No. 10808, 25 years, black. Generally contracted rachitic pelvis, D. C. 10.5 cm.

First pregnancy ended by an extraperitoneal Cæsarean section, after 24 hours of labor. Incision closed with catgut. There was a wound infection and the puerperium was febrile for six days. Second pregnancy terminated by a conservative Cæsarean section. Placenta posterior; uterine contraction fair; closure with catgut. The puerperium was febrile for seven days. The third pregnancy was ended by a Porro section. There were dense adhesions between the abdominal wall and uterus and also about the site of the extraperitoneal section. The placenta lay posteriorly; puerperium normal.

Description of Specimen.—After hardening, the uterus measures $15 \times 12 \times 9$ cm. To the left of the present incision is the scar of the second operation. It is marked by a shallow depression on the outer surface and a much deeper one on the inner surface. The scar measures 25 mm. as compared with a thickness of 35 mm. in the adjacent uterine wall, a ratio of 1 to 1.4. On microscopical examination there is complete regeneration of the musculature at the site of the scar, but there is also a considerable increase in the fibrous tissue. There is no trace of the scar of the extraperitoneal operation.

CASE XIX.—House No. 10758, 26 years, black. Generally contracted rachitic pelvis, D. C. 10 cm.

First pregnancy terminated by a conservative Cæsarean section elsewhere. The patient was in bed with a discharging wound for two months following it. At the second section done at the onset of labor, the lower two-thirds of the uterus was broadly adherent to the old abdominal scar, and after the adhesions had been released such an extensive raw bleeding area was left that it was thought best to remove the uterus. The placenta was inserted on the posterior wall; puerperium normal.

Description of Specimen.—After hardening, the uterus measures $15 \times 12 \times 6$ cm. On the anterior surface is the ragged area referred to above. On cross-section, the scar of the previous operation is visible as an irregular whitish line joining funnel-shaped depressions on the outer and inner surfaces (Fig. 17). On microscopical examination

there is little to mark the line of any former incision, the muscle fibers running from side to side without break in continuity and with only very slight increase in the fibrous tissue.

CASE XX.—House No. 11047, 19 years, black. Generally contracted rachitic pelvis, D. C. 8.75 cm.

The first pregnancy was ended by a conservative Cæsarean section at the onset of labor. The placental site was incised, the uterus contracted firmly, and closure was with catgut. Puerperium normal except for a rise of temperature to 101° on the second day. In second pregnancy the patient was admitted 36 hours after the onset of labor with intrapartum infection. A Porro section was performed with eventual recovery after a stormy convalescence. Dense adhesions bound the anterior surface of the uterus to the abdominal wall. Placenta posterior.

Description of Specimen.—After hardening, the uterus measures $15 \times 12 \times 8.5$ cm. In the midline is a slight longitudinal depression which corresponds with the scar of the previous operation. Cross-sections show almost perfect healing. The thickness of the scar is 21 mm., that of the adjacent uterine wall 29 mm., a ratio of 1 to 1.4. Microscopical examination shows normal muscle running directly across the line of former incision, with no increase in fibrous tissue.

RUPTURED CÆSAREAN SCAR

The following is the clinical history and the description of the amputated uterus from the single instance in our series in which the scar of a previous Cæsarean section ruptured. Figs. 18 and 19 show the anterior surface and cross-section of the uterus.

CASE XXI.—House No. 7570, 24 years, black. Generally contracted rachitic pelvis, D. C. 9.5 cm.

The first pregnancy was terminated by a prolonged spontaneous premature labor. The second was ended by a Cæsarean section, seven hours after the onset of labor. The uterus retracted poorly, and the incision was closed with two layers of chromic catgut. The puerperium was febrile for 10 days.

The third pregnancy progressed normally to the seventh month. On November 29, 1915, the patient complained of much abdominal pain, and was seen in her home by the out-patient service. As she was not in labor, and as the result of the examination was unsatisfactory, she was instructed to report to the dispensary on the following afternoon. This she did, walking a distance of six or eight blocks each way. She returned the next day with slight vaginal bleeding and was admitted to the hospital. On examination the cervix admitted one finger easily, while just above the internal os is a firm, rounded tumor apparently projected from the posterior wall of the uterus into its cavity, so that a tentative diagnosis of myoma was made. The abdomen was slightly distended but not sensitive, and it was impossible to map out the fetus or to hear the fetal heart. Temperature normal, pulse 100.

Under the supposition that we had to deal with a myoma, which would interfere with the birth of the dead child, laparotomy was decided upon. When the abdomen was opened with a median incision, a small amount of bloody fluid escaped and the dead fetus surrounded by the placenta and membranes lay free in the abdominal cavity. The uterus was tightly contracted, with a large, jagged, irregular, blood-stained opening occupying its anterior wall. There was no bleeding, and what had appeared to be a myoma on vaginal examination was found to be the firmly contracted posterior wall of the uterus. The organ was then removed by supravaginal amputation, and the patient made an ideal recovery.

The interesting points in connection with the case are:

- (a) the impossibility of determining when the rupture occurred;
- (b) the trifling clinical signs connected with it;
- (c) the difficulty of diagnosis;
- (d) the absence of serious hemorrhage, and
- (e) the total absence of shock both before and after the operation.

Description of Specimen.—The uterus, after hardening, measures 10x8x6 cm. On its anterior surface, just to the left of the midline, is a jagged, irregular blood-stained opening 6 cm. in length. There are practically no adhesions. Upon cross-section, it is seen that the muscle had failed to unite after the first section, as is shown by the fact that the edges of the opening are smooth and show no sign of recent tear. Evidently the scar had been composed only of decidua and peritoneum, which had yielded when the uterine distention had become pronounced. Microscopical examination bears out this supposition, as the decidua is found to cover the entire inner surface of the rupture, and to extend up to the peritoneum, which is quite thick and scar-like. Nowhere is there any suggestion that muscle union had occurred. The placenta had been implanted anteriorly over the scar.

Unfortunately, in a small number of our repeated Cæsarean sections, which were followed by removal of the uterus, the specimen was lost or mislabeled. Likewise, in a number of instances the patient was sterilized at the subsequent section by resection of the Fallopian tubes, and consequently anatomical studies of the scar could not be made. The main

interest in such cases lies in the fact that they serve to demonstrate that the uterine scar was able to withstand the strain incident to a second or third pregnancy. Tables III and IV have been so arranged as to give the salient points in each case without making a detailed report necessary.

DELIVERY BY THE NATURAL PASSAGES, FOLLOWING CÆSAREAN SECTION

In 1904, Von Leuwen was able to find in the literature 32 instances in which a previous Cæsarean section had been followed by delivery through the natural passages, and since then Brodhead, J. T. Williams, Willson, Harrar, Davis, Breitstein, Mason, Humpstone and others have reported similar cases. Study of their material shows that pelvic dystocia had only occasionally afforded the indication for the original operation, but that it had generally been undertaken for some such temporary complication as eclampsia or placenta prævia. Naturally, this is what one would expect, for had the pelvic contrac-

TABLE III

OUTLINE OF THE CLINICAL HISTORY OF PATIENTS WHOSE UTERUS WAS LOST, OR OF PATIENTS STERILIZED AT SECOND SECTION BY RESECTION OF THE FALLOPIAN TUBES

Case number	First operation					Second operation			Remarks
	Indication	Placental site	Closure	Uterine contraction	Puerperium	Placental site	Adhesions	Puerperium	
1520	Pelvic dystocia.	Posterior.	Deep silk superficial catgut.	Not recorded.	Febrile.	Not recorded.	Moderate.	Febrile.	Uterus removed but lost. No record of scar.
1548	"	Anterior.	Deep silk superficial catgut.	Not recorded.	"	Anterior.	Not recorded.	Excessive bleeding. Patient died on table.
1611	"	Posterior.	Catgut.	Firm.	"	Anterior.	Dense.	Febrile.	Death on 8th day, infection. Uterus secured at autopsy but lost.
5132	"	"	"	"	"	Posterior.	"	Afebrile.	First operation in Pittsburgh. No admissions after 2d section.
6787	"	Anterior.	Catgut.	Not recorded.	Febrile.	"	"	Febrile.	No subsequent admissions. No note of scar.
8242	"	Not recorded.	"	Not recorded.	Afebrile.	"	Moderate.	Afebrile.	Tubal sterilization. Old scar not seen.
8303	"	Anterior.	"	Firm.	"	"	Dense.	"	Tubal sterilization. No mention of old scar.
8537	"	Anterior.	"	Not recorded.	"	Anterior.	Not recorded.	"	Tubal sterilization. No mention of old scar.
8666	"	Posterior.	"	Poor.	"	Posterior	Filmy.	"	Tubal sterilization. Old scar not seen.
9169	"	"	"	"	Febrile.	"	Filmy.	"	First operation extraperitoneal. No subsequent admissions.
9282	"	"	"	"	"	"	Not recorded.	"	First operation done elsewhere. Tubal sterilization. Old scar-depressed line 5 cm. long. No subsequent admissions. Old scar not seen.
9547	"	Posterior.	Catgut.	Not recorded.	Afebrile.	"	Filmy	Febrile.	First operation in Washington, D. C. No subsequent admissions. Old scar not seen.
10129	"	"	"	"	"	"	Dense.	Afebrile.	No subsequent admissions. Old scar not seen.
10212	"	Posterior.	Catgut.	Poor.	Febrile.	"	Filmy.	Afebrile.	No subsequent admissions. Old scar visible as thin depressed line.
10779	"	Anterior.	"	Good.	Febrile.	"	Dense.	Febrile.	No subsequent admissions. Old scar visible as thin depressed line.
11031	"	Posterior.	"	Poor.	Afebrile.	Anterior.	Dense.	Afebrile.	No subsequent admissions. Old scar not seen.

TABLE IV

OUTLINE OF TWO CASES IN WHICH TWO CÆSAREAN SECTIONS PRECEDED THE ONE IN WHICH TUBAL STERILIZATION WAS EFFECTED

Case number	First operation					Second operation				Third operation				Remarks
	Indica- tion	Placental site	Closure	Uterine contrac- tion	Puer- perium	Placental site	Uterine contraction	Closure	Adhe- sions	Puerperium	Placental site	Adhesions	Puerperium	
8955	Pelvic dystocia.				Anterior.	Firm.	Catgut.	Dense.		Afebrile.	Posterior.	Filmy.	Afebrile.	First operation else- where—extraperitoneal.
9254	Pelvic dystocia.	Anterior.	Catgut.	Not re- corded.	Febrile.	Posterior.	Not re- corded.	Catgut.	Filmy.	Febrile.	Anterior.	Dense.	Febrile.	Tubal sterilization. One of old scars visible as depressed line.

tion been sufficiently pronounced to demand a Cæsarean section at the first labor, those subsequent to it would not be likely to terminate spontaneously. Occasionally, however, even in such cases, the patient may not realize the gravity of her condition, and may fail to summon medical aid until labor has been in progress for a number of hours, when examination may show that the head has become sufficiently molded to permit its descent into the pelvis, and the labor may terminate spontaneously, or at most by a simple forceps delivery. Doubtless had most of these women been seen before the onset of labor, they would have been subjected to a repeated section, for in the presence of a definite degree of disproportion we should not allow a vigorous test of labor, although, as has already been pointed out, if the disproportion is slight, we may allow labor to progress under careful observation.

The points of interest in our 13 cases of delivery by the natural passages following a Cæsarean section are outlined in Table V. It will be noted that in 11 there was a varying degree of pelvic contraction, although in three it had not been sufficiently pronounced to furnish the indication for the previous

section. Moreover, in Cases 5959, 6927, and 8572 the child was so premature that the question of pelvic dystocia did not enter into consideration at the subsequent labor. They are of interest, however, from the fact that active labor pains persisted for 18, 15, 7.75 hours, respectively, and thus adduced additional proof of the ability of the well healed scar to withstand the strain of a moderately severe labor.

As 48 of our patients were subjected to one or more repeated Cæsarean sections, the 13 cases just mentioned, to which may be added four other deliveries elsewhere, indicate that 25 per cent of the women who have had a previous section possess a uterine scar sufficiently strong to withstand the strain of delivery through the natural passages. Naturally, this does not represent the entire truth, as it must be supposed that many of the uteri which were incised at the second or third section would have proven equally as strong had the disproportion not been so marked as to contraindicate any test of labor.

The gross mortality attending the repeated sections was 5.9 per cent. The death of one patient from hemorrhage was due to the inexperience of the operator; but the other two

TABLE V
OUTLINE OF THE CLINICAL HISTORY OF 13 CASES DELIVERED THROUGH THE NATURAL PASSAGES FOLLOWING A PREVIOUS CÆSAREAN SECTION

Name Number Color Age	Obstetrical history previous to operation	Time Place	Operation				Pelvis	Labor				Remarks
			Indication	Placental attachment	Closure of uterus	Puerperium		Date	Duration	Character	Size of child	
E. W. 2754 Black—34	1899. Podalic version.	1901. Johns Hopkins.	Pelvic dystocia.	Posterior wall.	2 layers. 1. Deep silk. 2. Chr. c. 4.	Normal.	G. C. rhachitic. D. C. 10½ cms.	1906	7½ hrs.	Podalic version. 2d stage 23 hrs.	3335 gm. B. p. 10 cm.	In 1905 delivered in another hospital by forceps.
E. H. 5253 White—25	1908. Abortion—4 months.	1909. Cleveland.	Pelvic dystocia.	Normal (?)	G. C. typical. D. C. 11 cms.	1912	6½ hrs.	Low forceps. 2d stage 1 hr.	3149 gm. B. p. 9½ cm.	
H. L. 5959 Black—26	1911. Johns Hopkins.	Pelvic dystocia.	Posterior wall.	2 layers chr. c. g.	Febrile.	G. C. rhachitic. D. C. 9½ cms.	1913	18 hrs.	Spontaneous. Premature.	1400 gm. B. p. 7½ cm.	Uterus removed after Cæsarean in 1914. Scar—Excellent. Wound healed per primam. Fever six days.
E. Y. 6927 Black—32	1913. Johns Hopkins.	Eclampsia rigid cervix.	Anterior wall.	3 layers chr. c. g.	Febrile.	1914	15 hrs.	Manual dil. of cervix from 5 cm. Mid forceps.	2080 gm. B. p. 8 cm.	Wound healed per primam. Fever two days.
M. G. 7295 White—32	Four labors. All spont. at term.	1913. Chicago.	Tonamia.	Normal. D. C. not reached.	1915	4 hrs.	Spontaneous.	2780 gm. B. p. 8½ cm.	
M. S. 7333 Black—19	1914. Johns Hopkins.	Pelvic dystocia. Large child.	Posterior wall.	2 layers chr. c. g.	Febrile.	G. C. typical. D. C. 11 cms.	1915	10 hrs.	Spontaneous.	2415 gm. B. p. 9 cm.	Wound healed per primam. Fever nine days.
L. E. 7538 Black—30	Two abortions. Spontaneous. One tubal abortion.	1912. Johns Hopkins.	Prolapsed cord. Cervix 2 cms. dil.	Anterior wall.	3 layers chr. c. g.	Febrile.	Flat rhachitic. D. C. 10½ cms.	1915	20 hrs.	Spontaneous.	3830 gm. B. p. 9½ cm.	Wound infection. Fever six days.
E. R. 8572 Black—20	1915. Philadel- phia.	Pelvic dystocia.	G. C. funnel. D. C. 11 cms. T. 1. 8 cms. Simple flat.	1917	7½ hrs.	Spontaneous. Frank breech. Premature.	1980 gm. B. p. — ?	
J. S. 9490 White—30	1909. Spont. Stillborn. 1910. Spont. Seven mos. Stillborn. 1911. Spont. Term. d. 6 weeks. 1914. Spont. Seven mos. Stillborn.	1915. Johns Hopkins.	Pelvic dystocia.	2 layers chr. c. g.	Febrile.	D. C. 10½ cms.	1918	9½ hrs.	Mid forceps. 2d stage 14 hrs.	2620 gm. B. p. 8½ cm.	Extra peritoneal Cæsarean. In 1916 spont. at 7 months. Private doctor. Fever six days.
E. M. 9555 White—32	1917. Washing- ton.	Funnel pelvis.	Febrile.	G. C. funnel. D. C. 11½ cms. T. 1. 7½ cms.	1918	33 hrs.	Spontaneous.	3290 gm. B. p. 8½ cm.	Patient said she had "milk-leg." In bed many weeks.
M. T. 16583 Black 21	1918. Johns Hopkins.	Eclampsia rigid cervix.	Posterior wall.	2 layers chr. c. g.	Febrile.	G. C. typical. D. C. 11 cms.	1920	9½ hrs.	Low forceps. 2d stage 1½ hrs.	3234 gm. B. p. 8½ cm.	Wound healed per primam. Fever for eight days.
E. M. 16917 White—26	1917. Church Home, Baltimore.	Eclampsia.	Normal. D. C. not reached.	1921	27 hrs.	Induced— Bougie. Convulsions. Spontaneous.	2450 gm. B. p. 8½ cm.	In 1919 spontaneous at term.
E. C. 16993 Black—19	1917. Spontaneous at term. Difficult labor. Child died from intracranial hemorrhage.	1919. Johns Hopkins.	Pelvic dystocia.	Posterior wall.	2 layers chr. c. g.	Febrile.	G. C. rhachitic. D. C. 9½ cms.	1921	4 hrs.	Spontaneous.	2750 gm. B. p. 8½ cm.	Wound healed per primam. Fever for nine days.

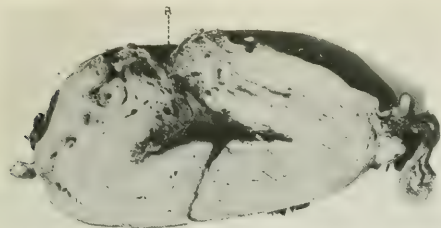
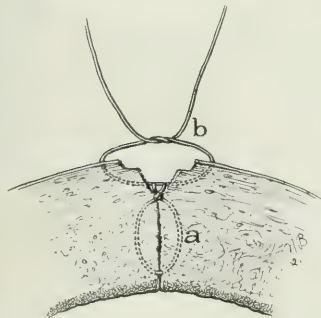
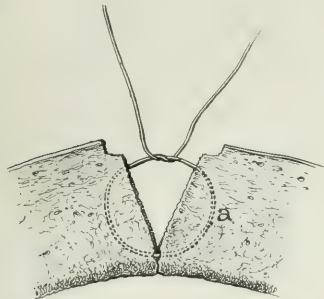
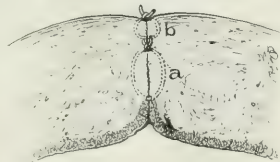
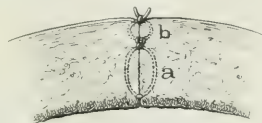


FIG. 1.—Crosssection of uterus removed at autopsy from patient dying from peritonitis (gas bacillus) on third day after operation. Case XI. (A) Site of recent incision. $\times 23$.



FIGS. 2 and 3.—Illustrating the two layers of sutures employed in closing the uterine incision. (A) Buried layer. (B) Superficial layer.



FIGS. 4 and 5.—Illustrating a possible cause of imperfect scars. In 4 the sutures have been tightly tied in a relaxed musculature. In 5 contraction has taken place with resulting indentations on both uterine surfaces.

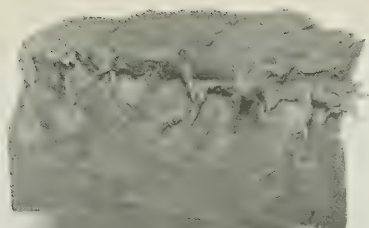


FIG. 6.—Scar from Case I, showing original silkworm gut sutures *in situ* at second section. $\times 1$.

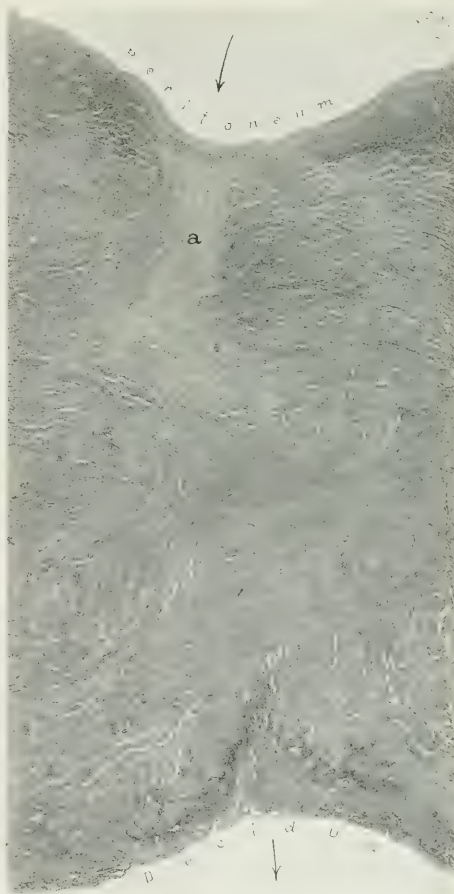


FIG. 7.—Drawing of cross-section of a comparatively well healed Cesarean scar. It demonstrates the typical depressions on both surfaces of the uterus and the regenerated muscle fibres, with almost no fibrous tissue. $\times 6$.

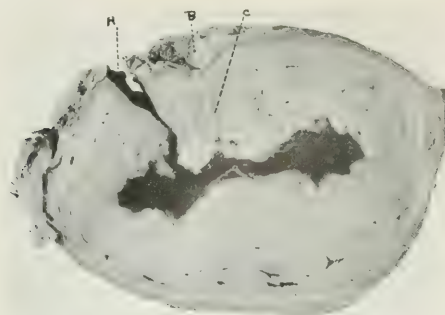


FIG. 8.—Cross-section of uterus from Case I. $\times \frac{3}{4}$. (A) recent incision. (B) Dense adhesions covering the old scar (C).

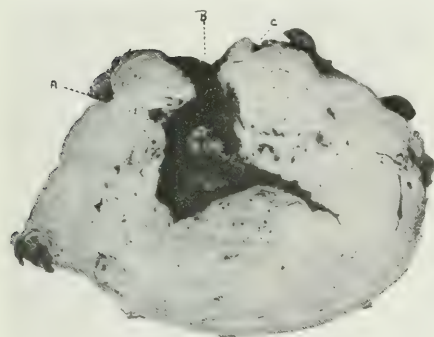


FIG. 9.—Cross-section of uterus from Case II. (A) Old scar. (B) Recent incision. (C) Old scar. $\times \frac{3}{4}$.

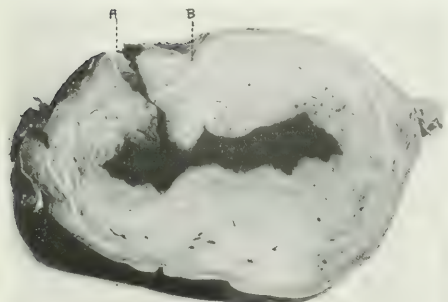


FIG. 10.—Cross-section of uterus from Case III. (A) Recent incision. (B) Old scar. $\times \frac{3}{4}$.

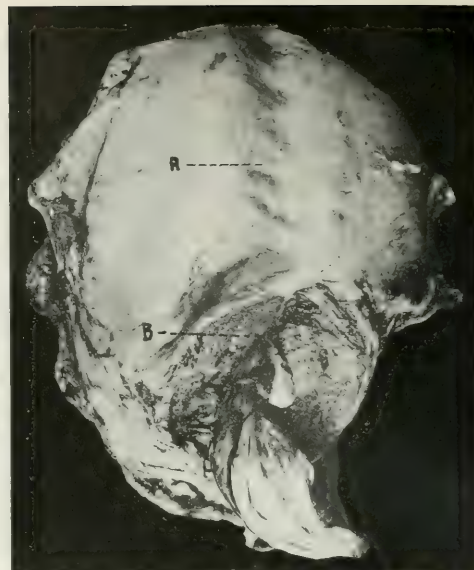


FIG. 11.—Uterus from Case XIII, showing (A) old scar with transverse indentations, and (B) recent incision. $\times \frac{3}{4}$.

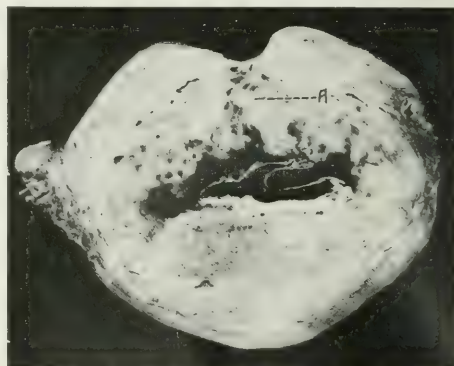


FIG. 12. Cross-section of uterus from Case XIII. (A) Old scar. $\times \frac{3}{4}$.

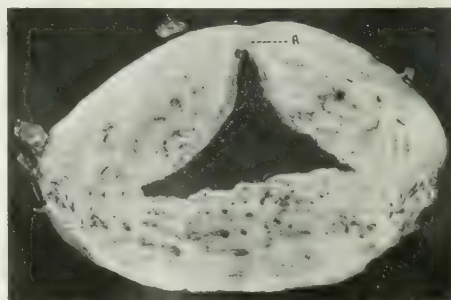


FIG. 13.—Cross-section of uterus from Case XIV. (A) Old scar. $\times \frac{3}{4}$.

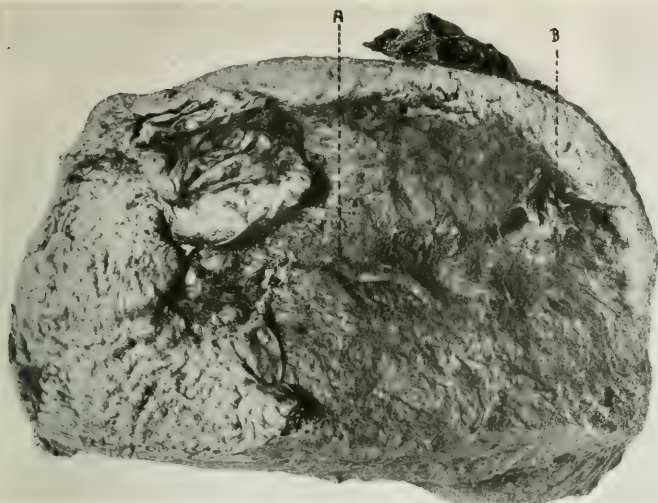


FIG. 14.—Inner surface of uterus from Case XVII. (A) and (B) upper and lower angles of old scar. $\times \frac{2}{3}$.

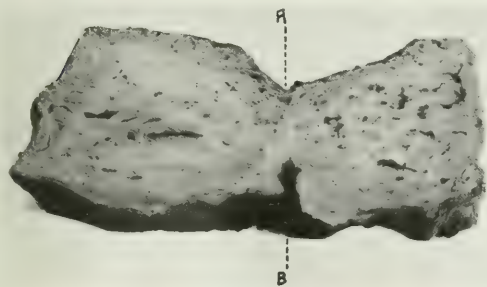


FIG. 15.

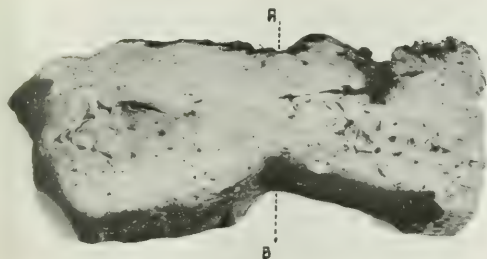


FIG. 16.

FIGS. 15 and 16.—Opposite sides of block 1 cm. in thickness from uterus in Case XVII. Shows variation in diameter of scar at various levels. $\times 1$.

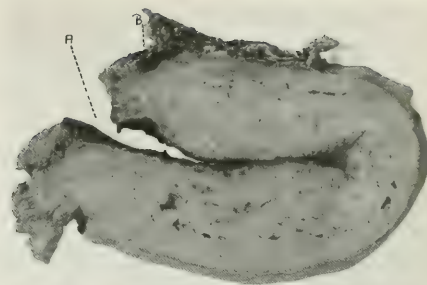


FIG. 17.—Cross-section of uterus from Case XIX. (A) Recent incision. (B) Old scar. $\times \frac{2}{3}$.

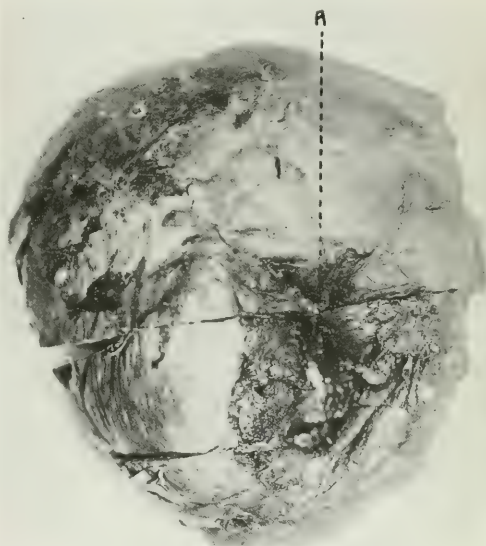


FIG. 18.—Uterus from Case XXI, showing the rupture of old Cesarean scar. $\times 1$.

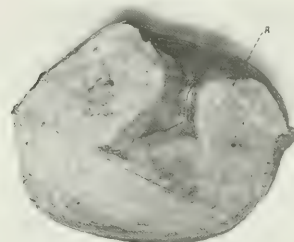


FIG. 19.—Cross-section of uterus from Case XXI. Note the smooth edges of the muscle at the site of rupture. $\times \frac{2}{3}$.

fatalities were directly attributable to the repeated abdominal operation. Our 48 women went through 63 pregnancies, 13 of which ended by vaginal delivery under our supervision, and 4 elsewhere, with only one instance of rupture of the uterine scar—an incidence of 1 to 63 or 1.5 per cent. It might be argued that this unusually low incidence is due to the fact that we are accustomed to sterilize our patients at the third operation, instead of doing five or six repeated Cæsarean sections, as is the practice of some obstetricians. Doubtless this may play some part in reducing the incidence of ruptured scars, as there can be no question that the greater the number of Cæsarean sections done upon one woman, the greater will be the number of imperfect scars. Consequently, we feel that, after a patient has been subjected to the risk of three major operations, she should be relieved of the added danger of a ruptured scar, which must inevitably become increased after each subsequent operation.

CONCLUSIONS

1. The weak Cæsarean scar may be due to a single factor or to a combination of factors, the most important of which is infection.
2. An afebrile puerperium does not give an absolute assurance of perfect wound healing.
3. The perfection of technique in suturing the uterine incision will undoubtedly lessen the incidence of weak scars.
4. Chromic catgut, in our hands, has proved to be a satisfactory suture material.
5. The uterine wound should not be closed, if possible, until firm contraction of the musculature has occurred.
6. As a rule foetal elements do not invade the uterine scar.
7. Adhesions following Cæsarean section are common. They are not necessarily the result of coexisting infection, and may give rise to serious complications at subsequent operations.
8. The dictum "once a Cæsarean, always a Cæsarean" cannot be accepted without considerable reservation.
9. A patient who has once been subjected to a Cæsarean section should enter the hospital several weeks prior to the expected date of confinement, so that she may have the benefit of immediate operation should rupture occur.

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ACUTE LOBAR PNEUMONIA AND HÆMATOGENOUS PUERPERAL INFECTION

A CASE REPORT

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The demonstration of a fixed type pneumococcus as the causative agent of a puerperal infection developing during the course of acute lobar pneumonia is unusual, and makes the following case report one of considerable interest both to the obstetrician and internist.

SUMMARY OF CASE.—*Thirty-five year old primipara; difficult labor with prolonged second stage; chloroform anæsthesia; low forceps delivery; immediate development of signs of acute lobar pneumonia; pneumococcus (type 1) septicæmia; pneumococcus (type 1) endometritis; anti-pneumococcus (type 1) serum therapy followed by rapid disappearance of organisms from the blood stream; prolonged course suggesting "delayed resolution"; recovery from endometritis; serum sickness; suggestive evidence of empyema; terminal hæmolytic streptococcus septicæmia; no autopsy.*

CASE REPORT.—A mulatto housewife, 35 years old, was admitted to the Obstetrical Service February 27, 1921. The family history was negative. The general health of the patient had always been poor. When a child she had had measles, mumps, chicken-pox and possibly pneumonia. At the age of 13 she had recovered after two months from an illness characterized by severe diarrhoea with tenesmus, blood and mucus. She denied having had diphtheria, scarlet fever, rheumatic fever, malaria or typhoid fever. She had suffered from frequent colds and occasional attacks of epistaxis. "Sore throat" had been a frequent winter complaint, and had been usually associated with enlarged tender cervical glands. Six months before admission to the hospital she had suffered for a short time from pollakiuria and dysuria, which had disappeared promptly under medical treatment. Two months before admission to the hospital she had had bilateral suppurative inguinal adenitis, which had been cured by incision and drainage.

Menstruation had begun at the age of 13; it had been regular and of three or four days' duration. The flow had been normal in amount and accompanied by a moderate amount of pain. She had been married in April, 1920, and the last menstrual period had occurred in May, 1920.

Present Illness.—The patient was first seen by one of us in the prenatal clinic on January 11, 1921. At that time she was eight months pregnant with the child lying in the right-occipito-transverse position. The examination of the heart and lungs was negative. The systolic blood pressure was 110; diastolic 70. The incisions in the inguinal regions were healing satisfactorily. The urine was normal and the blood Wassermann reaction was negative. The pelvic measurements were normal. At subsequent visits to the prenatal clinic, on January 26 and February 8, her condition was regarded as satisfactory.

She was admitted to the obstetrical ward in the first stage of labor at 9 p. m. February 27, after having walked several city blocks in a drenching rain. As the temperature was normal and examination of the heart and lungs was negative, she was regarded as being in good condition. Labor progressed satisfactorily until 3 a. m., February 28, when the second stage began. The head soon reached the outlet in the right occipito-posterior position, but as it made very little advance during the next three hours, operative delivery was decided upon. Under chloroform anæsthesia a deeply asphyxiated child was delivered at 6.20 a. m. after a difficult low forceps (Scanlon) operation—the total duration of labor being 29 hours and 40 minutes. The child was revived by sensory stimulation, and weighed 3380 gm. After the repair of a vaginal laceration, the placenta was expressed from the vagina. Approximately 300 c. c. of blood were lost. The routine microscopic examination of the placenta revealed no abnormalities and a subsequent study of sections stained for bacteria was negative.

The patient left the delivery room in excellent condition. Two hours after delivery, the temperature was 99.4° F., but from then on it rose steadily until it reached 104° F. at 8 p. m. At that time the patient complained of slight pain in the right side of her chest. No chill had occurred, and there was no cough nor sputum, nor were the respirations accelerated. On examination of the lungs a soft pleural friction rub was heard over the right lower lobe, without other changes. Abdominal examination was negative. During the night the temperature fell to 102.5° F., but rose to 104.2° F. the following morning. At that time (March 1) the patient was quite toxic. The respiratory rate was 36 per minute but there was no cough. The breath sounds were slightly suppressed over the right lower lobe and an occasional fine rale could be heard. The abdomen was quite distended and sensitive, but there was no muscle spasm or evidence of fluid. A blood culture was made and 24 hours later was reported positive for the pneumococcus (type 1).

On March 2, the general condition was essentially unchanged. The temperature remained elevated and the patient was quite toxic. Frank signs of consolidation of the lower lobe of the right lung were present. Lochia for intrauterine culture were obtained (by Little's tube), and consisted of a few cubic centimeters of dark serosanguineous fluid. On microscopic examination numerous diplococci were seen, which by culture and by serological examination proved to be pneumococci (type 1). A urine culture, made on March 1, was sterile. The patient was transferred to the medical service March 2, when examination revealed a fairly well nourished tired looking mulatto woman with high fever, tachycardia, moderate cyanosis, and rapid, shallow respiration. There was no cough or sputum. The pulmonary signs were those of consolidation of the right lower lobe. There was

marked distention of the abdomen with considerable tenderness over its lower third, but no muscle spasm or evidence of fluid. Rectal examination revealed a large firm tender uterus. Blood pressure; systolic 125, diastolic 65.

BLOOD EXAMINATION

R. B. C. count.....	3,393,000
Hæmoglobin (Sahli)	67%
W. B. C. count.....	6,250
Differential W. B. C. count.....	(300 cells)
Pm. N.	91.4%
Pm. B.	00 %
Pm. E.	0.7%
S. M.	3.0%
L. M. and T.	2.6%
Myelocytes	2.0%
Myeloblasts	0.3%

With the exception of a trace of albumin and a few white blood cells the urine examinations were negative. The Wassermann reaction (blood) was negative.

Immediately following admission to the ward, anti-pneumococcus (type 1) serum treatment was instituted, as follows:

March 2, 1921, 200 c.c. antipneumococcus type 1 serum intravenously.
 March 3, 1921, 200 c.c. antipneumococcus type 1 serum intravenously.
 March 5, 1921, 100 c.c. antipneumococcus type 1 serum intravenously.
 March 7, 1921, 100 c.c. antipneumococcus type 1 serum intravenously.
 March 8, 1921, 100 c.c. antipneumococcus type 1 serum intravenously.

A quantitative blood culture was made (blood-agar plate method) before the first serum treatment, and showed 173 colonies per c.c. of blood, whereas a culture made eight hours later and prior to the second treatment contained but three colonies per c.c. Specimens of blood were likewise taken before the third (March 3, 1921) and subsequent treatments, but were all sterile:

There was no systemic reaction to the intravenous administration of the serum, and the usual chill, leukopenia and the subsequent abrupt leukocytosis did not appear. However, there was a gradual rise in the number of leukocytes and two days after the first injection they reached 18,000 per c.mm.

With the termination of the septicæmia the patient's general condition improved greatly. The toxæmia became much less evident, and the abdominal distention less distressing. However, the temperature remained elevated and the physical signs in the lungs were essentially unchanged. There was still a moderate degree of pelvic tenderness on abdominal and rectal examination, as well as a fairly profuse, greenish, muco-purulent vaginal discharge. On March 6 (three days after the disappearance of the septicæmia), lochia were obtained for a second intrauterine culture. Smears showed a moderate number of leukocytes and lancet-shaped diplococci, and pneumococci (type 1) were again demonstrated in pure culture.

For two weeks the patient's condition remained practically unchanged, with the exception of a moderate degree of serum sickness, which first manifested itself on March 11. The temperature remained elevated (102.5° F.-104° F.), and the pulse varied between 110-130. The leukocyte count was between 18,000 and 35,000 per c.mm. There was very slight cyanosis and only a moderate degree of abdominal distention. The tenderness over the lower abdomen became much less marked and the vaginal discharge diminished in amount. On March 14 a third intrauterine culture was made and proved to be sterile. It was the opinion of those attending the patient that the prolonged course of the pneumonia was a manifestation of "delayed resolution," and that the uterine complication no longer formed a part of the picture.

On March 16, examination of the lungs showed signs suggestive of encapsulated pleural fluid high in the right axillary region; while the X-ray showed "remains of consolidation at the right base with suggestion of encapsulated fluid in the right axilla." The same

equivocal physical and radiographic findings were present the following day. On that day, the 17th, the patient complained bitterly of headache. She was very weak and slept most of the time. Ophthalmoscopic examination showed slight oedema and hyperæmia of the discs, but no other changes. Kernig's sign and neck rigidity were absent, and the reflexes were normal. Nevertheless, a lumbar puncture was performed. Clear cerebrospinal fluid under normal pressure was obtained, and contained ten cells per c.mm. The colloidal gold curve, Wassermann reaction and tests for globulin were negative. The patient's general condition rapidly became worse. The temperature remained high, the rapid pulse became weak and thready, and drenching sweats occurred. Cyanosis became marked and delirium developed. A pelvic examination revealed no abnormalities, and exploratory thoracocentesis performed at several different sites in the right axilla and back was negative. A blood culture taken on the evening of this day (March 17) revealed the presence of an overwhelming streptococcus septicæmia—approximately 7600 colonies per c.c. of blood. The patient gradually sank into coma. The pulse and respirations became irregular, and she died on the evening of March 18, the nineteenth day after delivery. Unfortunately permission for an autopsy could not be obtained.

DISCUSSION

The case presents several interesting clinical aspects. The sequence of events seems relatively clear. A primipara, 35 years old, with a past history of numerous infections, and on the whole of rather poor health, entered the hospital for delivery. At the onset of labor she was exposed for a short time to inclement weather. Labor was completed by forceps delivery under chloroform anesthesia. Immediately following the birth of the child an acute lobar pneumonia developed. As evidence of the lowered resistance of the patient to infection at this time, one might cite the absence of chill and leukocytosis at its onset, and particularly the existence of the septicæmia with large numbers of bacteria in the blood stream (173 colonies per cubic centimeter). Intrauterine culture demonstrated the presence of the same organisms in the post-partum uterus. That this finding was not accidental, or due entirely to the normal oozing of blood infected with pneumococci, is clear; for a greenish mucopurulent uterine discharge developed, which contained numerous white blood cells and organisms. Furthermore, this condition persisted for several days after the disappearance of the bacteræmia, and was accompanied by clinical evidence of uterine infection. Accordingly, it seems clear that we were dealing with a metastatic pneumococcus (type 1) puerperal endometritis, which developed in a woman suffering from acute lobar pneumonia.

When one considers the anatomical and physiological conditions present in the fresh post-partum uterus, one is struck by the fact that it presents a *locus minoris resistentiae*, as Bondy has aptly pointed out, and offers an ideal site for the growth of bacteria seeded into it by a heavily infected blood stream. In our case the puerperal infection obviously did not spread, but remained localized to the interior of the uterus, and had entirely disappeared clinically and bacteriologically by the 14th day. The disappearance of the type 1 pneumococcus bacteræmia following administration of the specific anti-pneumococcus type 1 serum in this case conforms in every respect to the observations of Avery, Chickering, Cole and Do-

chez, and others. That the serum had a beneficial effect upon the local intrauterine infection seems probable; for the process, although caused by a virulent organism and occurring in a patient with obviously lowered resistance, was in itself of rather benign type and of short duration. We feel therefore, that the use of the serum gave the patient a much better opportunity to overcome the infection, as was evidenced by the disappearance of the bacteræmia, the markedly improved general condition, the leukocytosis, the failure of the pneumonic process to spread to other lobes of the lung, as well as by the disappearance of the endometritis.

In concluding the discussion of the case from a clinical point of view, we feel that we may speculate with a fair degree of certainty concerning the terminal process. Although there was suggestive evidence, both from physical findings and radiographic examinations, that an empyema was developing and the prolonged course of the disease confirmed this possibility—repeated exploratory punctures of the pleural cavity failed to substantiate such a surmise. The normal cerebrospinal fluid almost conclusively rules out meningitis as a complication, and there was no evidence of the existence of pericarditis or endocarditis. The pelvic examination made the day before death was normal and the lochia negative. In the absence of a post-mortem examination, we accordingly have to fall back upon the assumption that we were dealing with a patient whose powers of resistance were such that she could not, in the usual length of time, overcome the local pulmonary infection, and as a result of the general debilitation resulting from it became a fit subject for a terminal generalized hæmolytic streptococcus infection, the respiratory tract serving as the portal of entrance. Moreover, it seems likely that the streptococcus pulmonary infection engrafted itself upon the slowly resolving pneumococcus pneumonia, causing an exacerbation of the pulmonary condition and resulted in an overwhelming generalized infection. MacCallum (4) who noted the occurrence of hæmolytic streptococci in three cases of lobar pneumonia studied in army camps, in which pneumococci had been isolated from the blood during the life or from the lungs at autopsy, has suggested the possibility that the latter organisms may have acted as the predisposing agents to infection with the streptococcus.

PNEUMOCOCCUS PUERPERAL INFECTION

The infrequency with which the pneumococcus is encountered as the etiological factor in puerperal infection no doubt explains, to a great extent, the interest with which instances of this condition have been regarded. Bondy, in 1912, published an excellent article upon the subject, which included a report of his own cases and a review of the literature. It is necessary to recall, however, that until comparatively recently the differentiation of the pneumococcus from certain strains of non-hæmolytic streptococci was fraught with difficulty, and that even to-day occasional strains are met with in which the differentiation is not sharply defined. It is, therefore, suggested that statistics compiled before the more recent advances

in bacteriological diagnosis were made should be accepted with some reservation, particularly as concerns the reported instances of "primary infection"—or infection by pneumococci gaining access to the uterus by way of the vagina.

It is manifest that two routes are open for the introduction of organisms into the uterus: (1) hæmatogenous (metastatic or secondary), (2) vaginal (primary). We shall consider each in some detail.

Hæmatogenous Pneumococcus Puerperal Infection.—Aufrecht, in 1884, was the first to produce an experimental pneumococcus endometritis by the introduction of living organisms into the blood-stream of rabbits shortly after labor. At autopsy a localized uterine pneumococcus infection was found with but insignificant changes elsewhere. Thus he proved experimentally that the puerperal uterus in that animal is a *locus minoris resistentiæ*, and that the pneumococcus is capable of producing pathological lesions in it. Orthmann (referred to by Bondy) confirmed this work. Weichselbaum, in 1888, reported the first instances of hæmatogenous pneumococcus infection of the puerperal uterus in women. Both occurred in patients suffering from acute pulmonary infections (exudative pleurisy and pneumonia). At autopsy the pneumococcus was found in the endometrium, as well as in the primary focus in the respiratory system. Bondy refers to the instance reported by Bumm in 1899, in which a pneumococcus peritonitis was associated with pneumonia and empyema. At autopsy pneumococci were demonstrated in the uterine blood vessels and secretions. Burchardt, in 1901, reported an instance of premature labor during the course of acute lobar pneumonia, in which pneumococci were found in the placental site and lymphatics of the uterus. Furthermore, Foa and Bordoni-Uffreduzzi cite two instances of abortion in pneumonic women in which pneumococci were found in the uterine veins. Stanoskiadis, in 1913, reported 12 cases of lobar pneumonia in which pneumococci were found in the vaginal secretions: He considered that they represented instances of hæmatogenous infection which was favored by the changes incident to abortion or delivery. Halper, Oertel and others have likewise discussed the subject.

In the light of the above reports, as well as of our own case, it seems profitable to recall that in 728 cases of acute lobar pneumonia reported by Cole, in which blood cultures were made, 27.8 per cent were positive for the pneumococcus. In other words, he demonstrated that a pneumococcus bacteræmia is probable in approximately every fourth case of pregnancy which is complicated by lobar pneumonia. Under these conditions the possibility of a pneumococcus infection of the uterus at the time of, or shortly after, its evacuation does not seem remote, and should be borne in mind by obstetrician and internist alike.

Vaginal (Primary) Pneumococcus Puerperal Infection.—Although our case obviously does not fall into this group, a brief survey of the subject may prove of interest. As has been intimated, our knowledge of the pneumococcus has been considerably extended during recent years, and the organism can now be more easily and accurately differentiated from other cocci. Allowances must, therefore, be made in interpret-

ing the results of investigators who worked prior to the acquisition of this knowledge.

It would seem that the pneumococcus is very rarely an inhabitant of the normal vagina. Bondy, in 1912, failed to find it in a search of the vaginal flora of 30 non-pregnant women, but found it once in the same number of normal pregnant women, as well as once in the lochia from 30 postpartum patients. Fabret and Bourret called attention to this work in 1913, and regarded it as affording conclusive evidence that the pneumococcus is not an inhabitant of the normal vagina. On the other hand, Schottmüller in 1910 found pneumococci in the lochia of three per cent of his cases of septic abortion; while Foulerton and Bonney in their examination of 54 puerperal women found pneumococci in four instances.

That the pneumococcus may occasionally be present in the vagina of the pregnant and puerperal women cannot be doubted, and in the light of the cases collected by Bondy and by Fabret and Bourret it seems certain that it may, under certain conditions, find its way into the postpartum uterus and there give rise to puerperal infection. The reader is referred to articles by the last named authors for an extended discussion of this question.

TREATMENT

Beside the general supportive measures usually employed in puerperal infections, Moore has suggested that uterine irrigations with ethylhydrocuprein (optochin), 1:10,000, may possibly be used to advantage, on account of the powerful antipneumococcus properties of the drug. If the infection is due to a type 1 organism, intravenous serum therapy may be indicated. In our case the uterine infection was of moderate degree so that local treatment was not attempted, but all of our efforts were directed to the relief of the pulmonary condition. However, we feel that the serum treatment was perhaps incidentally responsible for the mildness of the uterine infection and its early disappearance.

SUMMARY

We have reported a case of acute lobar pneumonia (pneumococcus type 1) with septicæmia as a complication of the puerperium, and have shown that a hæmatogenous (pneumococcus, type 1) endometritis developed and disappeared under observation. Death occurred on the 19th day from generalized hæmolytic streptococcus infection.

A brief review of the literature upon pneumococcus puerperal infections is given, and instances of both secondary (hæmatogenous) and primary (vaginal route) types are cited. It is suggested that the possibility of a pneumococcus puerperal infection should be borne in mind when dealing with patients in whom abortion or term delivery has occurred during the course of acute lobar pneumonia.

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DIPHThERIA BACILLUS CARRIERS

RESULTS OF RE-EXAMINATION OF APPARENTLY NEGATIVE CULTURES

By B. C. MARSHALL and C. G. GUTHRIE

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As ordinarily practiced, the examination of throat cultures for the presence of diphtheria bacilli is made after incubation of the tubes for from 12 to 24 hours, usually over night. Not infrequently a positive result may be obtained much sooner than this and in special cases examinations are made at intervals beginning at six hours, but it is quite generally the custom to discard as negative all cultures in which diphtheria bacilli have not been found after 24 hours in the incubator. This custom has probably arisen from several causes, as for instance:

1. The desire to secure positive information as early as possible.

2. The belief that the growth of the diphtheria bacillus on a suitable medium is relatively rapid during the first 24 hours, but that after that time it is apt to be overgrown by the other organisms encountered in cultures from the throat.

3. The belief that the morphology of the diphtheria bacillus is characteristic only during the earlier period of its growth on even the best of media; that after 24 hours its staining properties are lost to a greater or less degree and involution forms may appear, thereby rendering its recognition difficult or impossible in mixed cultures from the throat.

4. The belief that if diphtheria bacilli have not been found in the examinations of a throat culture during the first 24 hours they will not appear subsequently in that culture and therefore that search made at a later time would merely entail useless routine work.

We have no criticism to offer concerning examination of throat cultures "early and often," but we believe that the practice of regarding as negative all cultures which have not shown diphtheria bacilli up to 24 hours may yield results which are misleading, particularly in the case of bacillus carriers.

In the course of some experimental work with the so-called "elective medium" of Drigalski and Bierast,* we found that a number of the tubes, considered negative at 24 hours, were positive when re-examined a day later. It naturally occurred to us that the apparent delay in development of the diphtheria organisms might be due to the inhibitory effect of the bile in the special type of medium employed, but it soon became evident that a similar retardation might occur with other sorts of media as well. After this point was established we made it a rule to examine all cultures both at 24 and at 48 hours.

*v. Drigalski and Bierast: Nachweis der Diphtheriebazillen und seine praktische Bedeutung. Deutsche med. Wchnschr., 1913, XXXIX, 1237. This medium consists essentially of Loeffler's blood serum to which a certain amount of bile has been added prior to coagulation. The object of the addition of bile is to inhibit the growth of the other organisms from the throat without interfering with the growth of the diphtheria bacillus.

At this time we had under observation a series of diphtheria bacillus carriers—some of them healthy, others convalescent carriers, some who harbored virulent and others non-virulent organisms—and from these persons throat cultures were taken daily over a period of several weeks. The cultures were made on several different kinds of blood serum media and were examined with great care. All of the cultures found negative at 24 hours and almost one-half of the positive ones were re-examined at 48 hours. The results are presented in Tables I, II and III.

TABLE I
RESULTS OF CULTURES EXAMINED AFTER INCUBATION FOR 24 AND 48 HOURS

Cultures	Examined after incubation for 24 hours		Re-examination at 48 hours of cultures negative at 24 hours		Re-examination at 48 hours of cultures positive at 24 hours	
	Number	Per cent	Number	Per cent	Number	Per cent
Total.....	759	549	93
Positive....	210	27.66	52	9.47	92	98.92
Negative...	549	72.33	497	90.52	1	1.07

TABLE II
ANALYSIS OF POSITIVE RESULTS

Cultures	Number	Per cent
Total positives.....	262
Positive at 24 hours.....	210	80.15
Positive only at 48 hours.....	52	19.84

TABLE III
VIRULENCE OF DIPHTHERIA BACILLI IN CULTURES POSITIVE ONLY AT 48 HOURS

Medium	Virulent strains	Non-virulent strains	Total
Loeffler (pig serum).....	24	20	44
Loeffler (beef serum).....	1	1	2
Drigalski and Bierast (pig serum + bile).....	3	0	3
Drigalski and Bierast (beef serum + bile).....	3	0	3
Total.....	31	21	52

From Table I it is seen that of the 759 cultures from these carriers, 210, or 27.66 per cent, were positive at the first examination made after approximately 24 hours' incubation. Five

hundred and forty-nine cultures were negative at the first examination and of these 52, or 9.47 per cent, were positive when examined after an additional incubation of 24 hours. Re-examination of 93 originally positive cultures showed that 92 were still positive after 48 hours in the incubator.

An analysis of the positive results (Table II) shows that of the 262 cultures in which diphtheria bacilli were found, 210, or 80.15 per cent, were positive at the first examination, and that 52, or 19.84 per cent, were found positive only at the second examination which was made 24 hours later.

From Table III it is seen that among the 52 cultures found negative at the first examination but positive at the second, 31 contained virulent and 21 contained non-virulent strains of diphtheria bacilli. These 52 cultures were made on four different kinds of serum media, on all of which the diphtheria bacillus grows admirably when pure and, as a rule, also when it is present in mixed culture from the throat.

These results speak for themselves and require little comment. From them it is evident that practically one-fifth of the total number of positive cultures were regarded as negative when examined at 24 hours, but were shown to contain diphtheria bacilli when examined a day later. In all instances the original slides were saved for re-examination in case the culture should prove positive at 48 hours. Careful search showed the original report to have been justified in almost every instance, but whenever this re-examination revealed any diphtheria bacilli in the 24 hour slides, the earlier diagnosis was changed and such cases are omitted from this series. It is perhaps unnecessary to add that tubes which showed little or no growth at 24 hours are not included, even though subsequent examination may have revealed the presence of diphtheria bacilli. The practice of examining throat cultures both at 24 and at 48 hours has been continued since these figures were collected and the additional experience of several years has shown the advantage of this procedure. Since introducing it in this laboratory we have isolated typical Klebs-Loeffler organisms from 75 or more of these "delayed positives," thereby confirming the diagnosis originally based on morphology alone. The difference in morphology at 48 hours is practically negligible, being, when it occurs at all, in the nature of an increase in the number and size of the Babes-Ernst granules, thereby facilitating recognition of the organisms when the Neisser or similar contrast stains are used. In general, the development of involution forms seems to be less marked in mixed cultures from the throat than in pure cultures.

In our experience the "delayed positive" culture has been found only among carriers, healthy or convalescent, and has been quite independent of the virulence of the strain of organisms present in the throat. In cases of clinical diphtheria we have practically always found the organisms abundant and their recognition easy in throat cultures incubated for 24 hours, so that no difficulty exists from the source we have mentioned in the bacteriological diagnosis of actual cases of the disease.

The explanation of the "delayed positive" culture is far from clear. Whether the diphtheria bacilli present in cul-

tures from the throat of carriers may have a more prolonged period of lag than those from patients in the acute stage of the disease we are not prepared to say. In many instances this is obviously not the case, as the cultures may show at the first examination an abundant growth consisting almost exclusively of diphtheria bacilli. The organisms present in the "delayed positive" cultures, moreover, show a normal rate of growth when isolated in a pure state. We can only suggest that this peculiar retardation may possibly be due to the inoculation of the culture with a very small number of viable diphtheria bacilli and a very large number of other organisms which temporarily overgrow or possibly inhibit the former. Almost invariably, when Klebs-Loeffler bacilli were found to be sparse at 24 hours, they were abundant at 48 hours; if originally numerous, they were much more plentiful on the following day. In only one instance was a culture which was positive at 24 hours found negative at 48 hours. When once the diphtheria bacillus starts to grow, it apparently is able to hold its own against most of the other bacteria encountered in throat cultures with the exception of the hay bacillus and certain similar organisms. Many cultures have been examined after periods of growth longer than two days, but no evident advantage was found in this procedure.

Similar observations have been made by other workers. Knebel* collected some interesting statistics at the Hygienic Institute in Frankfurt; the results which form the basis of his report have been condensed and are shown in Table IV.

TABLE IV
RESULTS OF EXAMINATION OF THROAT CULTURES AFTER INCUBATION FOR ONE DAY AND TWO DAYS
(From Knebel)

Source of cultures	Result after incubation for one day		Result after incubation for an additional day		
	Negative	Doubtful	Negative	Doubtful	Positive
Fresh diphtheria suspects.	...	62	43	7	12
Convalescents	...	56	23	17	16
Fresh diphtheria suspects.	159	..	151	7	1
Convalescents	576	..	470	51	55

From Knebel's figures shown in Table IV, it is seen that of the throat cultures from 118 cases—62 fresh suspects and 56 convalescents—all of which were recorded as doubtful at the first examination, 28 were definitely positive, 66 were definitely negative and only 24 were still considered doubtful when examined a day later.

Table IV also shows that of 159 throat cultures from diphtheria suspects regarded as negative at the first examination, only one was found positive after incubation for another day and in this isolated instance both the history and the physical examination of the patient suggested strongly that they were

* Knebel, Max: Beiträge zur bakteriologischen Diagnose und Statistik der Diphtherie. Inaugural Dissertation, Giessen, 1912.

dealing with a convalescent rather than a fresh case of diphtheria. Further information is available concerning four of the seven cases which were reported as doubtful at the second examination; the organisms from two of these were shown not to be diphtheria bacilli; attempts to isolate the organisms seen in the culture from another were unsuccessful; the pure culture obtained from the fourth proved to be avirulent on animal inoculation.

Conditions were quite different, however, with regard to the 576 negative cultures from convalescents; 55 or 9.54 per cent, proved to be positive when examined next day and these findings were confirmed by further study including virulence tests on the organisms obtained in pure culture. The originally negative cultures from 51 cases which were moved to the doubtful column as the result of the second examination, Knebel dismisses with the comment that a doubtful result is of much less importance in a convalescent than in a diphtheria suspect and that, when dealing with convalescents, one cannot go wrong provided prophylactic restrictions are not relaxed on the basis of a doubtful answer.

Neisser,* who was director of the Frankfurt Institute speaks favorably of Knebel's report and recommends that (1) all cultures showing a poor growth after incubation for one day and (2) all throat cultures from convalescents should be re-examined on the following day.

Seidel† also lays stress on the desirability of examining all throat cultures from convalescents both at 20 and at 44 hours. He cites an instance in which failure to re-examine a culture which was negative at 20 hours resulted in the discharge of a convalescent child who promptly infected her three little sisters.

* Neisser, M.: *Bakteriologie der Diphtherie*. Centralbl. f. Bakteriöl., 1913, Section 1, LVII, Supplement, p. 1. Neisser, M., and Gins, H. A.: *Ueber Diphtherie*, in *Kolle and Wassermann's Handbuch der Pathogenen Mikroorganismen*, Ed. 2, Jena, Gustav Fischer, 1913, V, 931.

† Seidel, Otto: *Zur Behandlung der Diphtherie*, München. med. Wehnschr., 1915, LXII, 1209.

A comparison of our results with those reported by Knebel is presented in Table V.

TABLE V
COMPARISON OF OUR RESULTS WITH THOSE REPORTED BY KNEBEL

Source of cultures	Cultures negative at 24-hour examination	Cultures positive when examined at 48 hours	
		Number	Per cent
Diphtheria convalescents (Knebel)...	576	55	9.54
Bacillus carriers, convalescent or healthy (Marshall and Guthrie).	549	52	9.47
Total	1125	107	9.51

From Table V it is seen that our figures agree very closely with those reported by Knebel, although his observations were made entirely upon patients convalescent from diphtheria, whereas ours were made upon bacillus carriers, some of them convalescent but others healthy carriers.

From these results it seems evident that, when dealing with throat cultures from diphtheria carriers, the customary laboratory practice of making single or repeated examinations within the first 24 hours may not yield all of the information available and may lead to erroneous conclusions. The error from this source alone, as judged from the results in 1125 cases, is about 9.5 per cent. If one is interested in determining the duration of the carrier state, in ascertaining the actual condition of affairs, rather than in merely securing some arbitrary number of successive negative cultures required by health regulations prior to discharge of a patient, further investigation is essential.

It is to be recommended, therefore, that in the study of diphtheria bacillus carriers, either healthy or convalescent, all cultures which are negative up to 24 hours be re-examined after another day in the incubator.

THE HYDROGEN-ION CONCENTRATION OF TISSUE GROWTH IN VITRO

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INTRODUCTION

Before undertaking a study of the effect of bacteria upon growth of tissue culture, it seemed necessary to determine in what way media of different H-ion concentrations influence the activity of tissue cultures, and whether there is a parallelism between what we know of the determining factor of H-ion concentration on the life process of micro-organisms and on cultures of embryonic tissue. Numerous observers have shown that there is both an optimum H-ion concentration for bacterial growth and a limiting or final concentration at which

growth activity is at rest, both conditions being influenced by the constituents of the medium. It was our purpose, therefore, to determine the optimum and the limiting or final H-ion concentration for tissue cultures, and to show any possible similarity of metabolism between pure type embryonic connective-tissue cells and cellular organisms (bacteria) as grown *in vitro*. At the present the comparison must of necessity be crude, both because of the different conditions under which the two types of living cells exist and because of our meager knowledge concerning the elements necessary for life and propagation of cells.

TECHNIQUE

Explants of chick embryos in Locke-Lewis solution furnished a satisfactory means of following the hydrogen-ion concentration during the growth of tissues, because of the ease with which a medium of a given H-ion concentration can be prepared and its fluidity at all ages of growth, in contradistinction to plasma. After some experimentation, a colorimetric method was devised (Felton, 1921) by which it was possible to test out the small hanging drop of the tissue culture. This method is not only very simple but also apparently quite exact. A drop of indicator of approximately the same size as the hanging drop was placed directly upon the growth on the cover-slip, shaken or stirred about and, with an abrupt blow, shaken off into a white glass plate. An exact reading of the drop was then obtained by combining a drop of indicator with a drop of a known buffer solution until the same color as that of the hanging drop was obtained.

Tissue cultures were prepared in the usual manner (Lewis and Lewis, 1915). Locke-Lewis solution (85 c. c. of NaCl 0.9 per cent plus KCl 0.042 per cent plus CaCl_2 0.025 per cent plus NaHCO_3 0.02 per cent plus 15 c. c. chicken bouillon plus 0.25 per cent dextrose) has a H-ion concentration between 6.6 and 7, usually 6.8. When a medium of a given H-ion concentration was to be prepared, the sodium bicarbonate and dextrose were omitted and sodium hydroxide or hydrochloric acid was added until the solution, when tested after boiling for 15 minutes, gave the color corresponding to that of the buffer solution of the required H-ion concentration. To this was then added sufficient dextrose to make 0.25 per cent. Solutions of H-ion concentrations varying from 4.4 to 9.2, with an increment of 0.2, were used. Explants were made from connective tissue from chick embryos ranging from 5 to 14 days' incubation, but unless otherwise stated the results refer to tissue from embryos of 7 to 9 days' incubation.

The buffer solutions used in these experiments were furnished by Doctor W. Mansfield Clark. The indicators were thymol blue, methyl red, brom thymol blue, brom cresol purple, phenol red, cresol red, and phenolphthalein.

VARIABILITY OF THE H-ION CONCENTRATION OF LOCKE-LEWIS SOLUTION ON DIFFERENT KINDS OF COVER-SLIPS

A few series of cultures sufficed to show that care must be taken in selecting the cover-slips for these experiments. With cover-slips made from certain kinds of glass the control hanging drops, after incubation, varied greatly and many of them became so alkaline that the results of any experiments with different media would have been obscured. All cover-slips were placed in dilute sulphuric acid for one or more days before preparing them for use. Covers of three unknown makes, which had been in the laboratory a number of years, were the most satisfactory. When a drop of a solution of a known H-ion concentration was incubated upon these it remained almost unchanged for a number of days, so that after a period of three weeks in the incubator the H-ion concentra-

tion was approximately the same as that of the original solution (6.8 to 7.0). A number of other kinds of cover-slips were tested but proved to be unsatisfactory for our purpose, as the hanging drop sometimes changed within a few days from pH 6.8 to pH 8 or 8.6. It is clear, therefore, that the selection of cover-slips suitable for the purpose is of great importance before undertaking any experiments.

An attempt was made to render the unreliable cover-slips more satisfactory by coating them with celloidin or with paraffin. Those covered with a thin layer of celloidin were favorable for growth; the cells grew out as extensively and lived as long on such covers as on the usual ones, but the hydrogen-ion concentration of the control drops still varied greatly, due no doubt to the dialysis of the H-ion from the glass. It is possible that an impermeable membrane would produce almost ideal conditions. The alkalinity of these drops did not increase to such a marked extent as on the uncoated cover-slips, but the variations in the results with the individual cover-slips was so great as to render them undesirable for these experiments. On the cover-slips coated with paraffin the drops remained much more constant, but rounded up to such extent that such covers were useless for the purpose of tissue cultures. The limited number of reliable cover-slips obtainable at this time accounts for the small number of experiments given in the charts.

RANGE OF HYDROGEN-ION CONCENTRATION OF MEDIA IN WHICH CHICK TISSUE EXHIBITED GROWTH

Pieces of chick embryos were explanted into media having a H-ion concentration varying from 4 to 9.2, with an increment of 0.2. In solutions having H-ion concentrations of 4.0, 4.4, 4.8, 5.0 and 5.2 no growth was obtained, except in one series in which a few migrating cells wandered out from explants of a 68-hour embryo in a solution of pH 5.0. Many explants, from embryos of all ages, were made in solutions of pH 5.5, but none of these grew except in the case of a chick blastoderm of 24 hours' incubation. In general, the younger the embryonic tissue the greater the percentage of growth in media of low pH value.

Approximately one hundred cultures were explanted into solution with H-ion concentrations of 6.0, 7.0, 8.0 and 9.0. The percentage of growth which occurred in these cultures was respectively 71, 93, 89 and 81. In the normal Locke-Lewis solution, which has a H-ion concentration usually about 6.8, over two hundred cultures were explanted. Of these, 90 per cent grew. The greatest number of cultures grew in media of pH 7 and in the normal solution, and these cultures also exhibited the most extensive growth. It was frequently impossible to differentiate the series of cultures explanted into media having a H-ion concentration of 7.0, 7.2, and 7.4. What growth took place in solutions of pH 6.0 was extensive and healthy, but tissue from embryos of over 11 days' incubation seldom grew in this medium. The growth in solutions of pH 9.0 was frequently small, whereas that in solutions of pH 8.0 was more extensive and lived longer.

HYDROGEN-ION CONCENTRATION OF CULTURES GROWN IN MEDIA OF VARYING HYDROGEN-ION CONCENTRATIONS (pH 6, 7, 8, and 9)

Table 1 shows the results obtained when cultures explanted into solutions of different hydrogen-ion concentrations (pH 6.0, 7.0, 8.0 and 9.0) were tested by means of the drop colorimetric method described above. While the individual cultures varied greatly, there were no marked differences by which cultures belonging to one series could be distinguished from those of another. Cultures explanted into acid media (pH 6.0) were on the whole somewhat more acid than those in normal Locke-Lewis solution, while those explanted into alkaline media (pH 9.0) were a little more alkaline than those in neutral solutions.

The lack of any marked differences between the series of cultures was probably due largely to the fact that the explanted piece acted as a buffer, so that shortly after explantation the medium became changed to one more nearly neutral. This was especially so in the alkaline solutions. Cultures explanted into media, which, after explantation, had a H-ion concentration of 8.2 and 9.0, became within 3 to 4 hours pH 6.8 to 7.2 and pH 7.6 to 8.0, respectively. Not enough cultures were examined to justify any definite conclusions, but from the table it is evident that, regardless of the initial hydrogen-ion concentration of the medium, cultures which contained healthy and extensive growth tended to be neutral, those which failed to grow had usually become slightly acid, and those which had exhibited extensive growth and then degenerated were slightly alkaline. As will be shown later, however, this last point depends upon the amount of dextrose present in the medium.

It is difficult to understand what factors caused our results to differ from those obtained by Rous (1913), who studied cultures of chick embryo tissue explanted into plasma that had been colored blue with litmus. The differences in regard to healthy cultures may be explained on the ground that diffusion takes place much less readily in plasma than in Locke-Lewis solution; therefore, whatever acid was produced by the explant may have been held in the immediate vicinity of the explant and thus have become condensed to a sufficient strength to turn the blue litmus pink. This, however, does not account for the fact that in Rous's experiments cultures that failed to grow remained alkaline, while in ours such cultures were usually more acid than those exhibiting good growth. The varied results may be explained upon the basis of different media.

HYDROGEN-ION CONCENTRATION OF CULTURES GROWN IN LOCKE-LEWIS SOLUTION

The hydrogen-ion concentration of the solution used depended upon that of each preparation of bouillon. It was usually about 6.8, but in a few instances was 6.6, and in others 7.0. The amount of dextrose added to the solution varied, as it was measured, not weighed; supposedly about 0.25 per cent to 0.5 per cent dextrose was used. Two hundred and thirty-six cultures were tested in this solution (Chart 1).

Of these, 19 failed to grow, 107 exhibited extensive growth, and 110 were tested after the growth had degenerated.

The age at which the culture was tested had little bearing upon the hydrogen-ion concentration. The condition of the culture, on the other hand, appeared to have a marked influence, as it was found that cultures that had failed to grow were usually slightly acid. The greater number of those tested when the growth was extensive and healthy were neutral, with a range from pH 6.6 to 7.4; whereas, after degeneration had taken place, most of the cultures were slightly alkaline, ranging from pH 6.0 to 7.8.

INFLUENCE OF DEXTROSE IN THE MEDIUM UPON THE HYDROGEN-ION CONCENTRATION OF THE CULTURES

Tissues grew extensively in media containing from 0.25 per cent to 2 per cent dextrose. When dextrose was omitted from the solution, the cultures often exhibited an extensive cell proliferation within 24 to 48 hours, but soon became full of vacuoles, and degeneration took place within a few days (Lewis, 1921). When a large quantity (4 to 5 per cent) dextrose was added, the amount of growth was quite variable; sometimes large, again small, and in some instances consisting of only a few migrating cells. The cells of this growth, however, seldom contained any vacuoles, even after many days. The growths in media containing a large amount of dextrose usually lived longer (1 to 2 weeks) than those in media without dextrose (3 to 5 days), but seldom as long as those in cultures containing a smaller amount of dextrose (2 to 4 weeks).

It was difficult to determine the exact amount of dextrose most favorable for the cultures. It varied between 0.5 per cent and 1 per cent, depending upon the series; in some experiments, culture in a medium containing 0.5 per cent dextrose exhibited larger growth and lived longer than those in a solution to which 1 per cent dextrose had been added, while in other series the results were reversed. From an examination of the numerous cultures (over 500) explanted into media containing different percentages of dextrose, it was found that 0.25 per cent was seldom sufficient sugar to maintain a healthy growth for many days. The cells began to develop vacuoles within 4 to 5 days and died shortly afterwards.

Of the cultures explanted into media without dextrose, 71 were tested at death. Of these 19 had a hydrogen-ion concentration of 7, 34 were pH 7.2, 15 were pH 7.4 and 3 were pH 7.6. The longest period of life was 9 days; the greater number of the cultures died between the third and fifth day. Most of the solutions used as media had a hydrogen-ion concentration of 6.4 and 6.6; a few were pH 6.8 or pH 7.0. When dextrose was not added to the medium the cultures did not become acid during growth and degeneration. Those failing to grow, however, were slightly acid.

As shown in Table I, Chart I, and Table II, cultures containing 0.25 per cent to 0.5 per cent dextrose behaved in somewhat the same manner. On the other hand, cultures in media containing 1 per cent or more dextrose usually had become acid at death. The range of hydrogen-ion concentra-



FIG. 1.—A 7-day culture of connective tissue from an 8-day chick embryo. The dark area is the explanted piece and the distance from this to the cells at the top of the page may be used as an axis to indicate the extent of the outgrowth of cells which is meant by extensive growth. $\times 43$.

tion varied greatly in the different cultures. Usually, cultures that contained the most dextrose were the most acid. Those in 5 per cent dextrose (the largest amount of sugar used in these experiments) were frequently pH 5.8 to pH 6.0. The length of life of the growth in cultures containing more than

TABLE I
HYDROGEN-ION CONCENTRATION OF

The medium	The cultures after			
	3-4 hours	Failed to grow	Extensive growth	Large growth, later died
pH 6	5 were pH 6.4	3 were pH 5.8	5 were pH 6.4	2 were pH 6.0
	13 " " 6.1	17 " " 6.6	2 " " 6.2	" " 6.2
	12 " " 6.2	8 " " 6.8	2 " " 6.4	" " 6.4
	3 " " 6.4	1 was " 7.1	8 " " 6.6	" " 6.6
	1 was " 6.6		7 " " 6.8	" " 6.8
	1 " " 6.8		6 " " 7.1	" " 7.1
			8 " " 7.2	" " 7.2
			4 " " 7.4	" " 7.4
pH 7	10 were pH 6.8	2 were pH 6.2	1 was pH 6.6	1 was pH 6.1
	4 " " 6.4	5 were " 6.8	1 " " 6.8	" " 6.2
	3 " " 6.6	3 " " 7.1	1 " " 6.4	" " 6.4
	2 " " 6.8		7 were " 6.6	" " 6.6
			11 " " 6.8	" " 6.8
			7 " " 7.1	" " 7.1
			11 " " 7.2	" " 7.2
			7 " " 7.4	" " 7.4
			3 " " 7.6	" " 7.6
			1 was " 7.8	" " 7.8
pH 8	4 were pH 6.8	6 were pH 6.1	1 was pH 6.4	2 were pH 6.4
	6 " " 7.1	2 " " 6.6	9 were " 6.6	6 " " 6.6
	4 " " 7.2	1 " " 6.8	13 " " 6.8	7 " " 6.8
		1 was " 7.1	11 " " 7.6	7 " " 7.1
		2 " " 7.2	14 " " 7.2	11 " " 7.2
			6 " " 7.4	6 " " 7.4
			12 " " 7.6	12 " " 7.6
			5 " " 7.8	5 " " 7.8
pH 9	1 was pH 7.2	2 were pH 6.1	1 was pH 6.6	1 was pH 6.6
	2 were " 7.4	1 was " 6.8	2 were " 6.8	2 were " 6.8
	3 " " 7.6	1 " " 7.1	19 " " 7.1	7 " " 7.1
	5 " " 7.8	2 were " 7.6	8 " " 7.2	5 were " 7.2
	4 " " 8.1	1 was " 7.4	2 " " 7.4	2 " " 7.4
			8 " " 7.6	8 " " 7.6
			1 was " 8.1	1 was " 8.1

The hydrogen-ion concentration of cultures explanted into media having different hydrogen-ion concentrations (pH 6.0, 7.0, 8.0, and 9.0). The cultures which failed to grow were usually tested after 48 hours, but in some instances not until after three or four days. The age at which the cultures exhibiting extensive growth were tested varied from 24 hours to 14 days.

The final hydrogen-ion concentration was obtained by testing cultures just after the cells had died or while one or two still remained alive. This occurred between four days and four weeks, usually at the end of 15 days.

1 per cent dextrose seemed to be dependent upon the rapidity with which the formation of acid took place. When death took place, the cells appeared quite different from those in 0.25 per cent to 0.5 per cent sugar, as vacuolization did not occur nor did the cells round up; instead they became coagulated, retaining their size and shape somewhat as skeleton forms. The cultures that died after a few days of growth had

already become acid (pH 6.0), while those that still exhibited good growth when tested, even after a much longer period of time (10-14 days), had not yet done so.

The final hydrogen-ion concentration of the tissue cultures depended more upon the amount of dextrose added to the

TABLE II

Media	No. of cultures	Final H-ion concentration of culture
Without dextrose pH 6.8	1	pH 7.1
" " " "	5	" 7.2
" " " "	2	" 7.4
" " " "	2	" 7.6
" " " "	3	" 7.2
" " " "	4	" 7.4
" " " "	3	" 7.6
Cultures in media without dextrose	20	Average final pH=7.35
With 1% dextrose pH 6.8	2	pH 6.1
" " " "	3	" 6.2
" " " "	1	" 6.4
" " " "	4	" 6.6
" " " "	1	" 6.1
" " " "	1	" 6.2
" " " "	4	" 6.4
" " " "	3	" 6.6
" " " "	1	" 6.8
Cultures in media with 1% dextrose	20	Average final pH=6.39

The final hydrogen-ion concentration of cultures explanted into media to which no dextrose was added and into media containing 1 per cent dextrose.

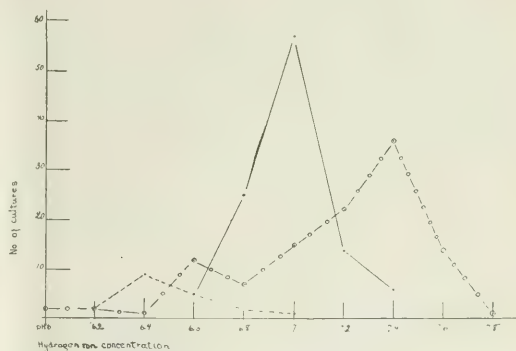


CHART I.—The hydrogen-ion concentration of 236 cultures explanted into normal Locke-Lewis solution. — (broken line) represents the hydrogen-ion concentration of the cultures which failed to grow; — (smooth line) that of cultures which were tested when the growth was extensive; o—o—o (dotted line) that of cultures which grew well but were not tested until they had degenerated.

Locke-Lewis solution than upon the original hydrogen-ion concentration of the medium. This is shown in Table II.

DISCUSSION

From the work reported in this paper it would seem that the optimum H-ion concentration for growth *in vitro* of embryonic connective tissue cells of a 9-day chick lies between 6.8

and 7.0. The question immediately arises, Why is the optimum H-ion concentration not 7.4, as it is in the blood of an adult chicken? Although not sufficient cultures were tried at a given pH to make it possible to decide this question, the wide range in which tissue cultures grew would make it appear plausible that growth can occur as well at pH 7.4 (same as blood) as at pH 6.8 to 7.0. However, it should be borne in mind that we are dealing with embryonic tissue, more capable, perhaps, of adapting itself to the experimental environment than are the adult cells. This may account for the wide range of H-ion concentrations in which the cells grow. As stated above, the younger the embryonic tissue the higher the percentage of growth in more acid media. Aggazzotti (1913) has shown that there is a gradual neutralization of the yolk as the embryo develops, changing from a pH 4.6 to a pH 6.4 from the first to the twenty-third day of its development. Granting this to be true, a 9-day chick embryo is developing in a more acid medium than an older one and in a less acid medium than a younger one. It seems reasonable that the optimum pH for a 9-day chick might be lower than for the adult animal and that this optimum would be the same as that of the yolk-sac at each respective stage of development.

The carbohydrate (dextrose) metabolism of embryonic connective tissue is seemingly very similar to that of bacteria. In a medium without dextrose, the pH increases as it does with most bacteria grown on ordinary dextrose-free medium, while with 0.25 per cent to 0.5 per cent of dextrose the H-ion level remains the same or swings a little to the acid or basic side from the original fluid. The two factors that influence this difference are the size of the drop of medium (buffer) and the condition and size of the explant. However, in a medium of 1 per cent dextrose, the entire buffer effect is used up by the acid produced from the dextrose, and the H-ion concentration is always more acid than the original solution. These results

are strikingly parallel to those found in the study of bacteria under similar conditions.

SUMMARY

Tissue cultures of chick embryos explanted into Locke-Lewis solution of a H-ion concentration between 5.5 and 9.0 exhibited growth. The medium most favorable for growth was one having a hydrogen-ion concentration about 6.8 to 7.0. The addition of dextrose to the medium was necessary for the healthy growth of cells over a period of time longer than three days. While all cultures in solutions containing up to 5 per cent dextrose exhibited growth, those in solutions containing between 0.5 per cent and 1 per cent had the greatest proliferation of cells and remained healthy for the longest period of time. Cultures that failed to grow were usually slightly acid, while cultures exhibiting extensive growth, when tested, were as a rule nearly neutral.

The final hydrogen-ion concentrations of the cultures depended upon the amount of dextrose in the medium. Those in solutions to which no dextrose had been added were pH 7.0 to 7.6, while those to which 2 to 5 per cent dextrose had been added were often pH 5.6 to 6.4.

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THE RELATION OF H-ION CONCENTRATION TO SPECIFIC PRECIPITATION

By V. R. MASON

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The purpose of this article is to present the results of a series of experiments designed to determine the relation of specific precipitation to the H-ion concentration of the solution in which the antigen-antibody reaction takes place.

The earlier experiments of Michaelis and Davidson¹ were made with combinations of sheep serum as antigen and immune rabbit serum as antibody. The H-ion concentration of the medium in which the precipitation reaction occurred was varied by the use of solutions of sodium acetate and acetic acid. Since non-specific protein precipitation occurred, their results were subject to an error dependent on the difficulty of

determining the amount of such precipitation, as may be readily observed in their protocols. They found that specific precipitation occurred between $[H] 3 \times 10^{-6}$ and $[H] 6 \times 10^{-6}$ when the antigen was employed in small amounts. If the dilution of antigen was less, however, precipitation occurred in the H-ion range of primary and secondary phosphate solutions, viz., from pH 5 to pH 9. They did not attempt to define the exact range of $[H]$ in which specific precipitation occurred.

In the series of experiments recorded below crystallized egg albumen was employed as antigen. A precipitating serum was produced in rabbits by three daily intravenous injections of 0.01 gm. of antigen in physiological salt solution. At the end of about three weeks the titer of the immune serum

¹ Michaelis and Davidson, Die Abhängigkeit spezifischer Fällungsreaktionen von der Wasserstoffionenkonzentration. Biochem. Ztschr., Berlin, 1912, XLVII, 59.

was usually greater than 1:100,000. The hydrogen-ion concentration of the mixtures of antibody and antigen was altered by the addition of various amounts of M/1 NaOH and M/1 H_3PO_4 . Since neither of these is a protein precipitant, confusion due to non-specific precipitation was avoided. Furthermore, wide variations of the hydrogen-ion concentration were possible. The NaCl concentration was kept constant by the use of 0.85 per cent NaCl as diluent for antigen.

PROCEDURE

Three parallel rows of 20 small test-tubes were set up and into each tube 0.1 c. c. of immune rabbit serum was introduced. The precipitin titer of the serum was greater than 1:100,000 in each experiment. 0.2 c. c. of a mixture of M/1 NaOH and M/1 H_3PO_4 was added to each tube in such a fashion that tube 1 of each series contained a solution whose pH was about 4 and tube 20 of each series contained a solution of about pH 10. The pH of the solutions in the intermediate tubes of each series ascended from 4 to 10 according to the typical curve. Antigen was next added as follows: To each tube of series 1, 0.1 c. c. of a 1:16,500 solution of egg albumen. To each tube of series 2, 0.1 c. c. of a 1:8250 solution. To each tube of series 3, 0.1 c. c. of a 1:1650 solution. The tubes were shaken and placed in a water-bath at 37° for 30 minutes. Certain controls were prepared as follows:

1. pH solutions plus solutions of egg albumen in the same amounts as used in the experiment.
2. pH solutions plus normal rabbit serum in the same amounts as used in the experiment.
3. pH solutions plus normal rabbit serum plus antigen in the same amounts as used in the experiment.

At the end of 30 minutes the results were read and the relative amount of precipitation noted. The pH of the tubes to right and left of the tube in which precipitation was just

visible was determined by colorimetric methods, the dyes recommended by Clark and Lubs being employed.

RESULTS

A series of experiments similar to those outlined above were performed and the results were fairly constant. Although there was occasionally difficulty in detecting minimum amounts of precipitation, the end-point was sharp unless the pH of the solution in adjoining tubes differed by less than 0.5. In general, precipitation was marked in the tubes which contained solutions whose pH ranged from 9.5 to 4.5 inclusive. The degree of dilution of the antigen was without appreciable effect unless this was greater than the precipitin-titer of the serum or low enough to be affected by the phenomenon of inhibition. Furthermore, although accurate methods for measuring the amounts of precipitation were not employed, there was apparently no more precipitate present in the tubes whose pH was near the isoelectric point of serum globulin (5.4) or of crystalline egg albumen (4.8).

In a further series of experiments similar to those outlined above, antigen and antibody were allowed to come in contact, with consequent precipitation, before the introduction of acid-alkali mixtures. The precipitate dissolved rapidly in the tubes which contained a mixture more alkaline than pH 9.5 or more acid than pH 4.5. In the tubes in which the H-ion concentration was between $10^{-4.5}$ and $10^{-9.5}$ inclusive the precipitate persisted, apparently unaltered.

CONCLUSIONS

Specific precipitation with the solutions employed above occurred between pH 4.5 and pH 9.5 inclusive. Moreover, specific precipitates permitted to form in a neutral medium were dissolved if the pH was reduced to less than 4.5 or increased to greater than 9.5.

NOTES ON NEW BOOKS

Ephraim McDowell (1771-1830), "*Father of Ovariectomy*" and *Founder of Abdominal Surgery, with an appendix on Jane Todd Crawford*. By AUGUST SCHACHNER, M.D., F.A.C.S., Louisville, Kentucky. (J. B. Lippincott, 1921.)

The discovery of vaccination against smallpox, the discovery of anesthesia, and the Listerian doctrine of antisepsis, were perhaps more dramatic in their presentation and reception by the world, but they were surely not more than coordinates in importance with McDowell's heroic effort in opening up the whole realm of abdominal surgery, which is the theme of an admirable life of Ephraim McDowell by August Schachner, of Louisville, Kentucky.

In this delightfully written work we have all the data for a complete history of one of our great medical pioneers, if not, indeed, the greatest. It has taken years of patient research to collect all the facts of the life of this great doctor frontiersman, and of his heroic patient, Jane Crawford, so that Schachner's work is conspicuous for its unflinching industry, and stands out as a model for future historians.

Our author is quite clear and is fully justified in pointing out that McDowell was not merely the first ovariectomist of the world, who thus

not only inaugurated a wholly new procedure, in which for a couple of generations he easily outstripped all competitors in his low death rate, but that he by this act of opening the peritoneum revealed the whole nascent realm of abdominal surgery.

Just one hundred and twelve years have passed since in December, 1809, Jane Crawford laid herself, a willing sacrifice, upon the table in McDowell's house, and endured the operation of cutting out a large ovarian tumor, confessedly an "experiment," repeating the Psalms as the doctor proceeded with his bold work. All precedents and the advice of all the eminent teachers of the time were against the innovation and, had it failed, what opprobrium would have fallen upon our progenitor's head; but since this rivulet started in the mountains of Kentucky, all the world has added its tributaries until it has become a mighty stream, destined still to enlarge and to flow on as long as man lives on earth and is subject to disease.

Europe at first disbelieved, then opposed, and then adopted the innovation, and last of all tried to rob the backwoodsman of his credit by faking an earlier operator. But to-day, McDowell's reputation is unassailable, and all rejoice to join with Schachner in placing laurels upon his worthy brow.

A further dramatic offset to the *mis en scène* is found in the objection to the operation raised by McDowell's nephew associate who lent only grudging aid in the rash procedure.

Alas, that we have so few such classics in our American medical literature as this excellent book. May it reach many of our doctors.

H. A. K.

The Oxford Medicine. By Various Authors. Edited by HENRY A. CHRISTIAN and SIR JAMES MACKENZIE. (Oxford University Press, London and New York, 1921.)

An inherently unfortunate feature of a system of medicine is this that for better or for worse it seems necessary to include all the diseases in the domain of the internist. The treatment of certain of the subjects, therefore, which are already covered by classical and altogether satisfactory monographs is foredoomed to inferiority. In the fourth volume of the Oxford Medicine one finds several sections which are excellent in themselves, but still suffer in comparison with previous well-known and readily accessible treatises. One may mention especially the chapters on the spleen, on the muscles, on the bones, and on sepsis. On the other hand, many of the articles are well worth while. Allbutt writes on gout in the older clinical style, leaving the chemical side to his associates; Longcope gives a brief but thorough summary of Hodgkin's Disease; Joslin on diabetes is always interesting, and Rountree summarizes the newer points of view in diabetes insipidus. The infectious diseases are well handled, but in a conventional way; the article on pneumonia by Irons is particularly valuable as a summary of the bacteriological side of this malady. The sections on industrial medicine are of interest, especially the introductory article on the physiology and pathology of work by Cecil and Katherine Drinker. On the whole the volume seems a useful one.

A. L. B.

JOHNS HOPKINS HOSPITAL BULLETIN

The Hospital Bulletin contains details of hospital and dispensary practice, abstracts of papers read and other proceedings of the Medical Society of the Hospital, reports of lectures, and other matters of general interest in connection with the work of the Hospital. It is issued monthly. Volume XXXIII is in progress. The subscription price is \$4.00 per year.

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Primary Carcinoma of the Liver. By M. C. WINTERNITZ, M. D. 42 pages. Price 75 cents.

The Statistical Experience Data of The Johns Hopkins Hospital, Baltimore, Md., 1892-1911. By FREDERICK L. HOFFMAN, LL.D., F.S.S. 161 pages. Price, \$2.00.

Venous Thrombosis During Myocardial Insufficiency. By FRANK J. SLADEN, M. D., and MILTON C. WINTERNITZ, M. D. Price, 75 cents.

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IMMUNOLOGICAL REACTIONS OF BENCE-JONES PROTEINS

II.—DIFFERENCES BETWEEN BENCE-JONES PROTEINS FROM VARIOUS SOURCES

By S. BAYNE-JONES and D. WRIGHT WILSON

(From the Departments of Pathology and Bacteriology and of Physiological Chemistry, The Johns Hopkins University.)

Since 1847, when Bence-Jones¹ described the peculiar substance now known as Bence-Jones protein, many preparations of it from different sources have been studied chemically. It has been well established that this substance is a protein with peculiar properties which render it unique among proteins. Furthermore, as the various preparations of this protein have been found to possess in general similar properties, and as the analyses of the specimens from two cases of Bence-Jones proteinuria made by Hopkins and Savory² agreed within the limits of their experimental error, there has been a

tendency to assume that all preparations of Bence-Jones protein are identical in structure and composition.

The usual determinations of the chemical constituents of proteins, however, have not afforded sufficient data on which to base an opinion as to the identity of those substances. As is well known, immunological reactions have indicated differences between proteins which were apparently alike. In some cases, as for instance in the comparison of the proteins of the eggs of the hen and duck made by Dale and Dakin,³ there has been found some correlation between the structure of the proteins

and their specific characteristics as antigens. As yet, no immunological comparisons of various preparations of the so-called "Bence-Jones protein" have been made.

Some aspects of Bence-Jones proteinuria suggest that the opinion that the substance has an invariable composition may not be justified. Bence-Jones proteinuria has been found in association with multiple myeloma, leukemia, carcinomatosis of bone, diseases of the kidney and other obscure conditions. Although the origin of Bence-Jones protein in the body is unknown, it is conceivable that the characteristics of the substance excreted in the urine might vary as the result of different pathological states. It is possible also that like the erythrocytes of man, and like bacterial proteins—those of the *Pneumococci* for example—the specimens of Bence-Jones protein excreted by different human individuals might have specific differences. The specimens of the protein are not all alike physically. There is certainly a great difference in their tendency to crystallize, or even to precipitate spontaneously; and varying temperatures of coagulation have been reported. Finally, the method of isolation of the protein from the urine might alter its physical, chemical and immunological characteristics.

The purity of the preparations has undoubtedly affected the results of immunological studies on the broad relationship of Bence-Jones protein to other proteins of human origin, particularly the proteins of human serum. Until recently, it was held that an animal immunized to Bence-Jones protein formed an antibody which precipitated human serum proteins. Massini⁴ and Hektoen⁵ have shown that by dilution and absorption of the antibodies in such sera it is possible with them to show a difference between Bence-Jones protein and human serum proteins. In a recent paper,⁶ we have described the results of an immunological comparison of Bence-Jones proteins with human serum. In this, we showed that a crystalline Bence-Jones protein acts as a single antigen, causing the production of strictly specific antibodies which do not affect the proteins of human serum. On the other hand, non-crystalline precipitated preparations of Bence-Jones protein were found to contain traces of human serum, which were responsible for the production of antibodies to human serum when these preparations were injected into animals. Aside from the effects attributable to contamination with traces of other proteins in the precipitated preparations, we think that the procedures, which will be described below, did not alter the fundamental antigenic qualities of the Bence-Jones proteins used in these experiments.

In a preliminary note,⁷ we reported that we had found immunological differences between various specimens of Bence-Jones protein. In this paper, we shall present in detail our evidence for the opinion that Bence-Jones protein is not a single substance, but that a group of similar, but not identical, proteins have been included in this designation.

LIST OF SPECIMENS OF BENCE-JONES PROTEIN
The various preparations of Bence-Jones protein which were used in this investigation were obtained as follows:

TABLE I.

No.	Patient	Diagnosis	Isolated by:	Method of isolation.
1.	H. M. R.	Bence-Jones proteinuria without demonstrable lesions in bones. No evidence of myeloma.	D. W. Wilson. Reported by Walters ⁸ .	Precipitated with sodium sulphate and acetic acid, washed and dried with alcohol and ether.
2.	H. M. R.	Do.	Do.	Coagulated by heating at 60° C. in slightly acid solution, centrifuged and dried with alcohol and ether.
3.	H. M. R.	Do.		Urine.
4.	H. M. R.	Do.	Do.	Crystallization.
5.	J. E. L.	Multiple myeloma?	A. Taylor ⁹ .	Precipitated like No. 1.
6.	F.	Multiple myeloma.	Guthrie and Boggs ¹⁰ .	Coagulated at 60° C. in solution made acid with acetic acid, filtered, dried over H ₂ SO ₄ .
7.	S.	Multiple myeloma.	Guthrie and Boggs ¹⁰ .	Coagulated, like No. 6.
8.	J. E. D.	Carcinoma with metastases to bones.	D. W. Wilson. Reported by Walters ⁸ .	Precipitated like No. 1.
9.	J. E. D.	Do.		Urine.
10.	R. L.	Multiple myeloma.	Do.	Precipitated like No. 1.
11.	R. L.	Do.		Urine.
12.	?	Multiple myeloma.	Rosenbloom ¹¹	Coagulated at 60° C.

The 12 preparations of Bence-Jones protein listed in Table I were derived from 5 patients affected with various diseases. Of these preparations, one (No. 4) crystallized spontaneously in the urine and was purified as completely as possible by recrystallization. In the comparison with human serum, which we have described, this protein acted as a single antigen. All the other preparations listed above contained traces of human serum proteins.

The immunological studies of these preparations were made by using precipitin, complement-fixation and anaphylactic reactions.

PRECIPITIN REACTIONS

The antisera to the Bence-Jones proteins used for the precipitin and complement fixation reactions were prepared as follows:

Rabbit No. 144—was immunized to the crystalline Bence-Jones protein No. 4 by 6 intravenous injections of a 1% solution of No. 4 at intervals of 4 to 6 days. The first dose was 2 cc., the last, 20 cc. Ten days after the last injection, when the rabbit's serum caused a precipitate in a 1 to 1,000,000 dilution of a 4% solution of No. 4, the animal was bled.

Rabbit No. 153—was injected intravenously at intervals of 4 to 6 days with amounts of a 0.5% solution of protein No. 12, increasing from 3 cc. to 20 cc. Six days after the fifth injection, the rabbit was bled. Precipitin titer: 1:100,000 (dilution of antigen).

Rabbit No. 189—was immunized to Bence-Jones protein No. 7. After 8 injections of amounts of 2 to 6 cc. of a 2% solution of this protein at intervals of 3 to 5 days, the precipitin titer of the serum was 1:2000 (antigen dilution.).

Rabbit No. 195—was immunized to Bence-Jones protein No. 6. At intervals of 6 days, 1 cc., 5 cc., and 7 cc. of a 3% solution of this protein were injected intravenously. Five days after the last injection, when the animal was bled, the precipitin titer of the serum was 1:8000 (antigen dilution.).

The precipitin tests were made in small clean sterile tubes with clear solutions of the antigens and clear sterile serum. Precautions were taken to eliminate false results due to the growth of bacteria in the mixtures. The Bence-Jones proteins were dissolved in salt solution with the aid of a small quantity of NaOH. The resulting solutions were neutralized with HCl until they were only very faintly alkaline to litmus. In the tests, the dilutions were made with normal saline. The pH of all dilutions above 1:100 was approximately 7. The various dilutions of the antigens were layered upon the undiluted antisera, and the first reading made by noting the presence or absence of precipitate at the plane of junction of the two fluids after they had been in apposition at room temperature for 1 hour. The fluids were then mixed, the tubes placed in the incubator at 37° C. for 24 hours, and a second note made of the sediment in the bottom of the tubes. Controls were made by mixing equal quantities of each component, serum or antigen, with salt solution and by incubating these mixtures with the tests. When precipitation occurred in the controls, the corresponding tests were discarded or repeated. The results of the precipitin tests are summarized in the following tables (Tables II, III, IV, V), in which the amount of precipitate is indicated by plus (+) signs.

TABLE II.

Precipitin reactions of antiserum to crystalline Bence-Jones protein vs. Bence-Jones proteins.

Serum 144
Anti—No. 4

Precipitation with Bence-Jones protein	Dilution of antigen						
	4%	1:10	1:100	1:1000	1:10,000	1:100,000	1:1,000,000
No. 1	++	++	++	++	+	0	0
2	+	++	+++	++++	+++	++	+
3 Urine	+	++	++	=	0	0	0
4	0	+	+++	++++	+++	++	+
5	++	0	0	0	0		
6	++	+	0	0	0		
7	+	0	0	0	0		
8	+	+	+	+	?		
9 Urine		+	+	=	0		
10	0	±	0	0	0		
11 Urine		+	0	0	0		
12	0	+	0	0	0		

TABLE III.

Precipitin reactions of antiserum to No. 6 vs. Bence-Jones proteins.

Serum 195
Anti—No. 6

Precipitation with Bence-Jones protein	Dilution of antigen				
	4%	1:10	1:100	1:1000	1:8000
No. 1	0	0	0	0	0
2	0	0	0	0	0
3 Urine	+	0	0	0	0
4	0	0	0	0	0
5	+	0	0	0	0
6	++	++++	+++	++	+
7	++	+	+	0	0
8	0	+	0	0	0
10	+	±	0	0	0

TABLE IV.

Precipitin reactions of antiserum to No. 7 vs. Bence-Jones proteins.

Serum 189

Anti—No. 7

Precipitation with Bence-Jones protein	Dilution of antigen				
	4%	1-10	1-100	1-1000	1-2000
No. 1	0	++	+	0	0
2	+	+	0	0	0
3 Urine	0	++	+	0	0
4	+	0	0	0	0
5	0	=	+	++	+
6	++++	++	+	+	0
7	++++	++++	+++	++	+
8	0	+	++	++	+
10	++	++	+	+	0

TABLE V.

Precipitin reactions of antiserum to No. 12 vs. Bence-Jones proteins.

Serum 153

Anti—No. 12

Precipitation with Bence-Jones protein	Dilution of antigen				
	4%	1-10	1-100	1-1000	1-10,000
No. 1	+	0	0	0	0
2	0	0	0	0	0
3 Urine	+	=	0	0	0
4	0	0	0	0	0
5	+	+	+++	+++	+
6	++	++	++++	+++	++
7	0	+	++	+++	+
8	0	0	+	=	0
9 Urine	+	++	+++	++	0
10	0	0	++	+++	+
11 Urine	+	+	++	++++	+
12		+	++	+++	+

The results of the precipitin reactions presented in Tables II, III, IV and V may be summarized as follows:

(a) The highly potent antiserum to the crystalline Bence-Jones protein (No. 4), having a titer of 1-1,000,000

for its homologous antigen, precipitated all the preparations of Bence-Jones protein. All, however, were by no means affected to the same degree. This antiserum showed a definite affinity for the preparations derived from the patient H. M. R., precipitating Nos. 2 and 4 to the end-titer, No. 1 in a dilution of 1-10,000, and a 1-1000 dilution of No. 3, which was this patient's urine. Proteins Nos. 8 and 9 were precipitated by this antiserum in dilutions up to 1-1000, while Nos. 5, 6, 7, 10, 11 and 12 were not precipitated when diluted above 1-50. As a consequence of its high titer, this antiserum demonstrated the group or class relationship of the various preparations, and at the same time indicated the sub-grouping among them. It is to be recalled that the preparation used to immunize the rabbit which produced this serum was free from human serum proteins. This antiserum did not precipitate human serum. The precipitin reactions with it, therefore, are strictly specific for Bence-Jones proteins.

(b) The antiserum to Bence-Jones protein No. 6, having a titer of 1-8000, showed no affinity for the isolated protein from patient H. M. R. (Nos. 1, 2 and 4). As preparation No. 6 contained a trace of serum proteins, its antiserum also precipitated human serum. To this is to be attributed the precipitate produced when it was added to No. 3, the urine from H. M. R., which contained some serum proteins. While this antiserum slightly affected the other preparations of Bence-Jones proteins, it did not precipitate any one of these in a dilution above 1-100.

(c) The antiserum to preparation No. 7 also included a precipitin for human serum proteins, as its antigen contained traces of these proteins. It caused precipitation with all the preparations of Bence-Jones proteins. The preparations from the patient H. M. R., however, (Nos. 1, 2 and 4) were precipitated only in concentrated solutions. On the other hand, proteins Nos. 5, 6, 8 and 10 were precipitated by this serum to its end-titer.

(d) As Bence-Jones protein No. 12 contained traces of human serum proteins, its antiserum precipitated human serum. This antiserum did not precipitate preparations Nos. 2 and 4, the coagulated and crystalline protein from patient H. M. R. Preparations Nos. 1 and 3 from the same patient, containing traces of serum proteins, were precipitated in concentrated solutions by the antiserum to No. 12. The other preparations were precipitated to almost the end-titer by this serum.

While this series of comparisons is not large, it indicates that there are antigenic differences between these

preparations of Bence-Jones proteins. Two distinct groups are recognizable, namely:

GROUP I.—Crystalline Bence-Jones protein No. 4, and other preparations, Nos. 1, 2 and 3 from patient H. M. R.

GROUP II.—Bence-Jones proteins Nos. 5, 6, 7, 8, 10 and 12.

In addition, there are certain less sharply defined differences between the proteins listed in Group II. For example, proteins Nos. 5 and 6 seem to be representatives of different groups.

An attempt was made to define these groups more sharply by applying the method of the absorption of precipitins. The conditions which at present limit the applicability of this method were discussed in our previous paper in relation to the removal of the antibody to human serum proteins from some of these antisera. The method was found to be unsatisfactory with the sera and solutions of proteins used in this investigation.

COMPLEMENT FIXATION REACTIONS

Tests were made to determine the ability of the antisera to the crystalline Bence-Jones protein No. 4 and to protein No. 12 to fix complement in the presence of the other Bence-Jones proteins. The sera were diluted with equal quantities of normal salt solution and heated to 56° C. for half an hour. To 0.25 cc. of each serum was added 0.25 cc. of a 1-20 dilution of a 4% solution of each Bence-Jones protein, or a 1-20 dilution of urine containing Bence-Jones protein. These dilutions were found by preliminary titrations to be beyond the limit of their anticomplementary action, except in the case of No. 2. They were, however, just within the range of the effective concentration for the occurrence of precipitation. These sterile mixtures were allowed to stand for 10 hours at 20° C. to permit precipitation to occur. At the end of that time, 0.25 cc. of a 1-10 dilution of fresh guinea-pig serum was added to each tube, and the tubes incubated in the water-bath at 37° C. for ½ hour. After this incubation, 0.25 cc. of antisherp red-corpuscle serum, containing 3 units of amboceptor, and 0.25 cc. of 2.5% suspension of sheep corpuscles were added, and the tubes returned to the water-bath. Readings of the presence or absence of hemolysis were made at the end of 1 hour, when all the controls showed the expected results. In the following table, in which the results of these tests are collected, the degree of complement fixation is indicated by plus signs (+).

TABLE VI.
Complement Fixation Reactions.

Serum	Bence-Jones antigen	Dilution of antigen	Results of fixation tests
Serum 144	No. 1	1-40	++
Anti—No. 4	2	1-20	anticomplementary
Dil. 1-2	3	1-20	++++
	4	1-20	++++
	5	1-20	++
	6	1-20	+
	7	1-20	0?
	8	1-20	++
	9	1-20	++
	10	1-20	+
	11	1-20	+
	12	1-20	+
Serum 153	No. 1	1-20	0
Anti—No. 12	2	1-20	anticomplementary
Dil. 1-2.	3	1-20	0
	4	1-20	0
	5	1-20	0
	6	1-20	++
	7	1-20	++
	8	1-20	+
	9	1-20	+
	10	1-20	+
	11	1-20	+
	12	1-20	++++

The complement fixation reactions show the general group relationship between the Bence-Jones proteins, and in addition they confirm the results of the precipitin reactions in demonstrating a great antigenic difference between Bence-Jones protein No. 12 and the preparations from patient H. M. R., represented by the crystalline protein No. 4.

ANAPHYLACTIC REACTIONS

The anaphylactic responses of guinea-pigs and of smooth-muscle preparations from these animals were used to continue the immunological analysis of the Bence-Jones proteins. Young females, weighing about 150 grams, were sensitized by an intravenous injection of 1 cc. of a 1.5% solution of some of the proteins. This injection was not followed by any toxic symptoms. After an interval of 18 to 21 days the second intoxicating injection was given intravenously, or uterine horns were excised from some of the sensitized virgin guinea-pigs and used to obtain graphic records of the anaphylactic contractions according to the method of Schultz and Dale.¹² Sensitization was easily effected by a single in-

jection of the proteins, except No. 4. For the experiments with this protein it was necessary passively to sensitize the guinea-pigs by an intraperitoneal injection of 1 cc. of serum 144, which contained precipitin specific for protein No. 4. Eighteen hours after this injection the animals were found to be hypersensitive to protein No. 4. The data of the experiments and the results of the anaphylactic reactions are summarized in Table VII and Figures 1, 2 and 3.

In interpreting the data of these anaphylactic reactions, it is to be borne in mind that all the preparations of

The anaphylactic reactions confirm the results of the experiments with precipitins and complement fixation. They show clearly that the protein from H. M. R., represented typically by the crystalline preparation No. 4, is distinct from the others, and that Bence-Jones proteins Nos. 5 and 12, while having some similarity, are probably representatives of other groups.

SUMMARY AND COMMENT

Twelve preparations of Bence-Jones protein obtained by various procedures from the urines of 5 patients, who

TABLE VII.
Anaphylactic Reactions.

Guinea Pig	Sensitizing dose of Bence-Jones protein	Interval	Intoxicating dose of Bence-Jones protein	RESULT
230	No. 1, 1 cc. 1.5% sol.	19 days	No. 1, 2 cc. 4% sol.	Typical shock, death in 1 min.
231	No. 1, 1 cc. 1.5% sol.	19 days	No. 4, 1.5 cc. 4% sol.	Slight reaction.
232	No. 1, 1 cc. 1.5% sol.	19 days	No. 5, 1.5 cc. 4% sol.	Shock. Death.
233	No. 1, 1 cc. 1.5% sol.	19 days	No. 8, 1.5 cc. 4% sol.	Shock. Death.
234	No. 1, 1 cc. 1.5% sol.	19 days	No. 10, 1.5 cc. 4% sol.	Shock. Death.
194	No. 5, 1 cc. 1.5% sol.	19 days	Nos. 1, 4, 6, 7, 8, 10, 12, 1 cc. each. No. 5. See Figs. 1 and 2.	Excised uterus. No contraction caused by Nos. 1, 4, 6, 7, 8, 10, 12. Strong contraction caused by No. 5.
293	No. 5, 1 cc. 1.5% sol.	19 days	No. 5, 0.23 cc. 4% sol.	Severe Shock.
294	No. 5, 1 cc. 1.5% sol.	19 days	No. 4, 1.5 cc. 4% sol.	No reaction.
296	No. 5, 1 cc. 1.5% sol.	19 days	No. 1, 1.5 cc. 4% sol.	No reaction.
270	No. 12, 1 cc. 1.5% sol.	19 days	No. 12, 0.75 cc. 4% sol.	Shock. Death.
271	No. 12, 1 cc. 1.5% sol.	19 days	No. 5, 1 cc. 4% sol.	Shock. Death.
272	No. 12, 1 cc. 1.5% sol.	19 days	No. 4, 1 cc. 4% sol.	No reaction.
273	No. 12, 1 cc. 1.5% sol.	19 days	No. 1, 1 cc. 4% sol.	No reaction.
274	No. 12, 1 cc. 1.5% sol.	19 days	No. 1, 1 cc. 4% sol.	No reaction.
5	Passively sensitized to No. 4	18 hours	1 cc. 4% solutions of Nos. 5, 6, 7 and 4. See Fig. 3.	No contraction caused by Nos. 5, 6, 7. Strong contraction followed by desensitization caused by No. 4.

Bence-Jones proteins, except No. 4, contained traces of human serum proteins. As we were unable to measure the amounts of serum proteins in the various preparations, we are unable to correlate the results of these reactions with that factor. The solutions of some of the preparations may have contained sufficient serum proteins to sensitize the animals to those proteins as well as to the Bence-Jones protein component of the mixture, while they may or may not have contained enough serum proteins to cause shock attributable to serum proteins in the intoxicating dose. The error due to the common effect of the presence of serum proteins would undoubtedly confuse the result, and would tend to emphasize the similarity of the preparations of Bence-Jones protein. Nevertheless, the differences exhibited by some of the proteins used in these reactions are so considerable that their significance in most instances is quite clear.

had Bence-Jones proteinuria associated with several different diseases were compared immunologically. Precipitin and complement fixation tests and anaphylactic reactions were studied with these preparations and the antisera obtained by immunizing rabbits to some of them. One of the proteins, No. 4, was crystalline and acted in this, as in a previous investigation,⁶ as a single antigen, free from serum proteins. The other preparations precipitated from urine by various means, contained traces of human serum proteins. As these preparations contained at least two antigens, one of which was not precisely measurable, some difficulties and confusion attended their use. Nevertheless, the results obtained in experiments with the crystalline protein were unequivocal, and most of the reactions with some of the other preparations were sufficiently clear for the purposes of this investigation.



FIG. 1.

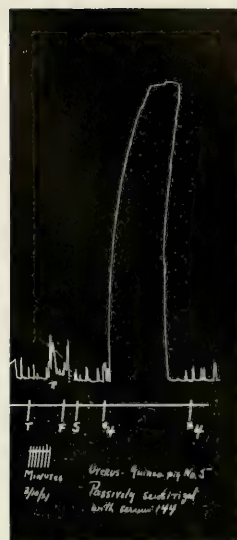


FIG. 3.

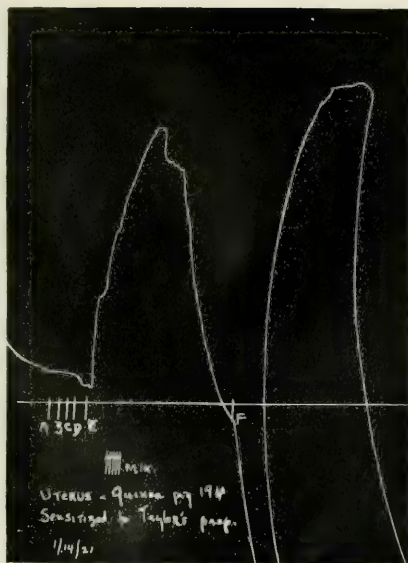


FIG. 2.

FIG. 1.—Uterus of guinea pig actively sensitized to Bence-Jones protein No. 5. Guinea pig 194.

At A: 0.5 cc. 4% Bence-Jones protein No. 4

At B: 0.5 cc. 4% Bence-Jones protein No. 1

At C: 0.5 cc. 4% Bence-Jones protein No. 12

At D: 0.5 cc. 4% Bence-Jones protein No. 5

FIG. 2.—Uterus of guinea pig 194 actively sensitized to Bence-Jones protein No. 5.

At A: 0.5 cc. 4% Bence-Jones protein No. 10

At B: 0.5 cc. 4% Bence-Jones protein No. 8

At C: 0.5 cc. 4% Bence-Jones protein No. 6

At D: 0.5 cc. 4% Bence-Jones protein No. 7

At E: 0.3 cc. human serum

At F: 2 cc. 4% Bence-Jones protein No. 5

FIG. 3.—Uterus of guinea pig No. 5.

Guinea pig passively sensitized to Bence-Jones protein No. 4 by intraperitoneal injection of 1 cc. of serum 144. Reaction tested 18 hours later.

At T: 1 cc. 4% Bence-Jones protein 5

At R: bath changed.

At F: 1 cc. 4% Bence-Jones protein No. 6

At S: 1 cc. 4% Bence-Jones protein No. 7

At 4: 1 cc. 4% Bence-Jones protein No. 4

Differences and similarities were demonstrated among these proteins. The methods used in isolating the proteins had no appreciable effect upon their immunological relationship, nor was there any obvious correlation between the types of the proteins and the diseases affecting the patients who excreted them. Of course, more specimens of Bence-Jones protein from various different pathological, and perhaps normal sources must be examined in this manner before it can be asserted positively that neither the disease with which the proteinuria is associated nor the method of isolation of the specimen determines the basic antigenic character of the protein. It is suggested, however, that such differences as exist may be related to the fundamental impress which an organism, bacterium or animal, places upon most of the proteins which are formed in its body.

From the immunological reactions of the preparations used by us, we are able to draw the following conclusions:

CONCLUSIONS.

1. Under the term "Bence-Jones protein" have been grouped a number of proteins which are similar but not identical.

2. Certainly two, and possibly three groups of Bence-Jones proteins are recognizable by immunological tests.

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STUDY ON EXPERIMENTAL RICKETS. XIX.

THE PREVENTION OF RICKETS IN THE RAT BY MEANS OF RADIATION WITH THE MERCURY VAPOR QUARTZ LAMP

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In a previous article¹ we showed that when rats were placed on a rickets-producing diet (diet 3143) they did not develop rickets if they were exposed to direct sunlight.

¹ The Prevention of the Development of Rickets in Rats by Sunlight. XI. P. G. Shipley, Dept. of Pediatrics, Johns Hopkins University, Baltimore, Md., E. A. Park, G. F. Powers, Dept. of Pediatrics, Yale University, New Haven, Conn., E. V. McCollum and Nina Simmonds, School of Hygiene, Johns Hopkins University, Baltimore, Md. *Proc. Soc. for Exper. Biol. and Med.*, Oct. 19, 1921.

STUDIES ON EXPERIMENTAL RICKETS. XIV. The Prevention of the Development of Rickets in Rats by Sunlight. P. G. Shipley, Dept. of Pediatrics, Johns Hopkins University, Baltimore, Md., E. A. Park, G. F. Powers, Dept. of Pediatrics, Yale University, New Haven, Conn., E. V. McCollum and Nina Simmonds, School of Hygiene, Johns Hopkins University, Baltimore, Md. *Jour. Am. Med. Assn.*, Jan. 21, 1922, Vol. 78, pp. 159-165.

In the résumé of the literature on the subject of light in relation to rickets which is introductory to that article, attention was called to the fact that radiation other than sunlight had been used to prevent or cure rickets in human beings. Buchholz used the "Glühlicht" which he stated was poor in chemically active rays; Huldshinsky, Putzig, Karger, Riedel, Erlacher and Mengert reported the use of the mercury vapor quartz lamp, and Winkler the use of the X-ray.

All the evidence as to the preventive and curative effects of the radiations from the mercury vapor quartz lamp in the rickets of human beings has been furnished by the X-ray. In order to determine the protective action of these radiations in experimental rickets in rats and also to examine the bones themselves we performed the following experiments:

Nineteen rats, mostly mixed black and white and about seven weeks old, were placed on diet 3143 which, as previous experience has shown, produces rickets comparable in every respect to the rickets manifesting itself in human beings. The ration has the following composition:

	Per cent
Wheat	33.0
Maize	33.0
Gelatin	15.0
Wheat Gluten	15.0
NaCl	1.0
CaCO ₃	3.0

It contains nearly twice the optimal content of calcium and is decidedly below the optimum in its content of phosphorus and of fat-soluble A. Otherwise it is well constituted.²

Nine rats were kept as control animals under ordinary laboratory conditions in a room completely screened with windows of ordinary glass. Ten rats were exposed to the radiations from a Hanovia mercury vapor quartz lamp (Alpine type).

One of the control animals (16Y) was found paralyzed thirty-eight days after being placed on the diet (age about eighty-eight days) and was killed. We have previously pointed out that the development of paralysis of the posterior extremities not infrequently occurs in rats fed on diet 3143. Another control animal (26Y) was killed fifty-eight days after being placed on the diet (age about one hundred and eight days); and the other seven animals were killed sixty-four days after being placed on the diet (age about one hundred and fourteen days).

The rayed animals were exposed to the radiations from a mercury vapor quartz lamp for varying periods of time for sixty-four days and were then killed. The animals were exposed to the radiations for two minutes on the first day; the period was gradually increased during the succeeding seven days to one hour daily; for the succeeding fifty-six days of the experimental period the animals were rayed for two to six hours daily, the length of time being determined largely by the availability of the lamp. The cage used was constructed so that animals could not be shielded in any way from the radiations. The floor of the cage was placed three feet from the quartz tube.*

The rayed animals never showed conjunctivitis, but

*STUDIES ON EXPERIMENTAL RICKETS. VIII. The Production of Rickets by Diets Low in Phosphorus and Fat-Soluble A. McCollum, E. V., Simmonds, Nina, Shipley, P. G., Park, E. A. *Jour. of Biol. Chem.*, Vol. XLVII, No. 3, August, 1921.

*We have found since the conclusion of these experiments that, before our use of the Alpine Lamp was begun, it had depreciated about eighty-five per cent (estimated) in its output of ultra-violet rays. This estimate was purely a guess made by a representative of the Hanovia Company, but of its essential truth we have no doubt. Prolonged exposure to the radiations of the lamp has not caused pigmentation of the skin in children.

early in the experiment spots of pigmentation on the ears of some of them were observed; this was especially marked in the albinos. This pigmentation was not present at the time the animals were killed. The albinos among the rayed animals showed slight yellow tinting of the hair on their backs.

As the experiments progressed, certain differences in the behavior of the two groups of animals were observed. The rayed animals were extremely active, the contrast between the two groups becoming more and more marked and reaching its maximum in the second third of the experimental period. Whenever any one came near the cage in which these animals were, a constant scurrying to and fro was almost always the striking feature observed. Not only were the control animals markedly less active, but their gait was distinctly waddling in character after the third or fourth week of the experiment. The disability of the control animals was well illustrated by the fact that toward the end of the experiments seven of the animals got out of their cage and were all recovered with relatively little difficulty.

The rayed animals always seemed to be hungry and, although the food given to each group was not measured, it was obvious that the amount consumed by the animals receiving mercury lamp radiations greatly exceeded that consumed by those in the control group. Increased appetite, then, was a striking characteristic of the rayed animals.

One of the rayed animals gave birth to six young on the thirty-seventh day of the experiments.

The autopsies revealed many differences in the animals. As shown in the following table, the measurements corroborate the observations made as to the better physical development of the animals exposed to the radiations from the mercury vapor quartz lamp.

	Control Animals (8)	Rayed Animals (9)	Per cent Increase in Rayed over Controls
Average Weight	140 grams.	157 grams.	12
Average Nose-Tail Length	31.6 cm.	34.5 cm.	9
Average Nose-Rump Length	15 cm.	16.1 cm.	7
Average Tail Length	16.6 cm.	18.4 cm.	10

The hair of the rayed animals was noticeably thicker and coarser than that of the control animals. The hair on the backs of the albino rats exposed to ultra-violet rays was tinted a slight lemon yellow. There was no enlargement of the epiphyseal junctions in the rayed animals. On opening the bodies of the rayed animals the most striking feature was the great amount of fat deposited in the subcutaneous tissue and in the peritoneal cavity. In some animals this deposition was tremendous. It occurred in some of the control rats but was never great. The muscular development of the rayed rats was better than that of the control animals. There were no deformities of the thorax and no gross evidences of rickets in the long bones of the rayed animals.

The control rat (16Y), autopsied thirty-eight days after the beginning of the experiment, showed the typical pic-

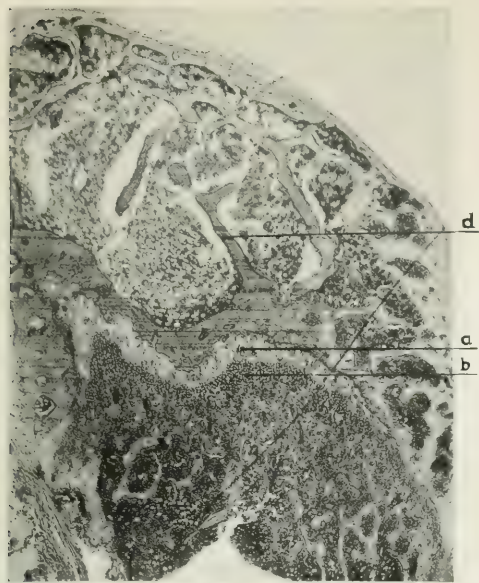


FIG. 1

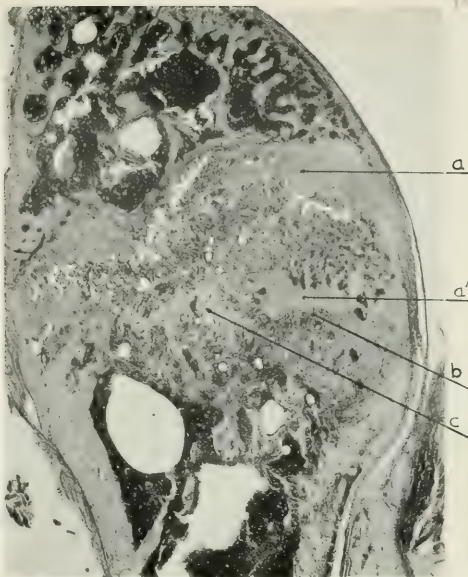


FIG. 2

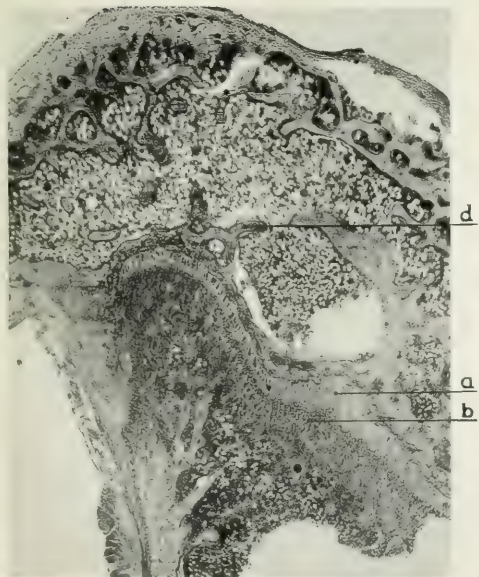


FIG. 3

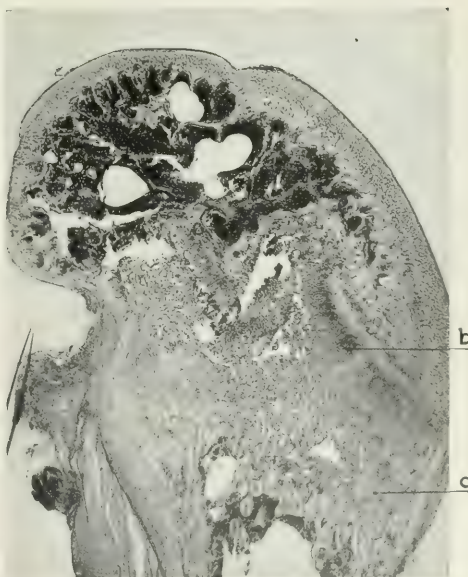


FIG. 4

ture of advanced rickets. The incisor teeth were brittle, the molar teeth were loosened; there was enlargement of the knees and wrists; the costo-chondral junctions were enlarged and bent inwards, and there were numerous fractures of the ribs. The tibiae and femora on section showed the typical gross picture of rickets.

The other control animals, autopsied about four weeks later, showed less marked but equally definite signs of rickets. The teeth were only slightly more easily fractured than normal. There was moderate enlargement of the epiphyses of the long bones of the extremities. The costo-chondral junctions were enlarged but there was little deformity of the chest and no well marked fractures of the ribs. The bones of the extremities cut with diminished resistance and in the femora of some there were gross evidences of rickets on section. The viscera showed no pathological changes. The amount of subcutaneous and peritoneal fat was much less than in the rayed animals. The muscles of the control animals were relatively poorly developed.

Microscopic examinations of the bones were confirmatory of the gross findings. In sections of the long bones of the rayed animals there were no evidences of rickets. The line of demarcation between the cartilage and shaft was sharp and clear cut. The deposition of calcium in the provisional zone of cartilage was heavy and complete. The trabeculae showed only the thin margin of osteoid which is seen in healthy, growing animals and which must be regarded as physiological.

The sections of the long bones of the control animals without exception showed rickets, but in some specimens the rachitic process was moderate in severity. The trabeculae in all sections were surrounded by broad osteoid borders and the calcification of the provisional zone of cartilage was fragmentary and exceedingly irregular. The degree of rickets usually found in rats on this diet is more severe than is shown in some of the specimens from these animals. This is probably explained by the fact that the animals were fifty days old when placed on the diet and were in some instances one hundred and fourteen days old when killed. During the period covered by the experiments, therefore, activity in growth was declining and with it there was a decline in the activity of the rachitic process.

DISCUSSIONS

From this experiment it is possible to say that rats fed on the rickets-producing diet (diet 3143) are protected from that disease by exposure to radiations from a mercury vapor quartz lamp. It is possible to say further that radiations from the mercury vapor quartz lamp affect not the skeleton alone but indeed the whole organism. While the development of a normal skeleton in the rayed animals is a "striking, visible and measurable" effect of the radiations on a single tissue, growth, good muscular development, storage of fat, improvement in the condition of the hair, stimulation of sexual development and

reproductive power are evidences that the radiations have a favorable influence upon the animals as a whole. So far as we are able to discern, the action upon rats fed the rickets-producing diet (diet 3143) of the radiations of a mercury vapor quartz lamp in securing an efficient utilization of the substances which are directly or indirectly concerned with ossification and calcification and in promoting general bodily vigor is in no way different in respect to these matters from the action of cod-liver oil and of sunlight.

SUMMARY

1. The object of the experiments was to determine whether or not radiations from a mercury vapor quartz lamp prevent the development of rickets in the rat.

2. A diet was employed which at room light regularly gives rise to a disease identical in its essential features with rickets as seen in the human being. The diet was high in calcium, low in phosphorus and was insufficiently supplied with fat-soluble A. In other respects it was well constituted.

3. Nineteen rats were placed on the diet. Ten were exposed to radiations from a Hanovia "Alpine" mercury vapor quartz lamp for varying periods of time daily over a period of sixty-four days. Nine rats were kept under conditions of ordinary room light as control animals.

4. One of the control animals was killed after thirty-eight days; another after fifty-eight days and the remaining seven after sixty-four days. All of these animals showed gross and microscopic evidences of rickets.

5. The ten rats exposed to the radiations from the mercury vapor quartz lamp were killed after sixty-four days. These animals were free from rickets both grossly and histologically.

6. The beneficial effects of the radiations from the mercury vapor quartz lamp were not limited to the skeleton, since the condition of the rayed animals underwent a general improvement.

7. The effects of the radiations of the mercury vapor quartz lamp on the growth and calcification of the skeleton of the rat and on the animal as a whole seem to be similar to, if not identical with, those brought about by direct sunlight and by cod-liver oil.

DESCRIPTION OF PLATES

Figs. 1 and 2. Microphotographs of sections of femora of rats fed on diet 3143 and exposed to radiations from a mercury vapor quartz lamp. Note sharp demarcation between shaft and cartilage at metaphysis (a), the heavy calcification of provisional zone of cartilage (b), and the wide bony trabeculae without osteoid borders (d).

Figs. 3 and 4. Microphotographs of sections of femora of rats fed on diet 3143 and kept at room light. Note wide irregular metaphysis.

Fig. 3. a—Proliferative cartilage.

à—Islands of cartilage with (b) deposition of calcium on the diaphyseal side.

c—Osteoid trabeculae.

Fig. 2. b—Cartilage is irregular and uncalcified.

A GRAPHIC METHOD FOR THE CALCULATION OF DIABETIC DIETS IN THE PROPER KETOGENIC-ANTI-KETOGENIC RATIO

By R. R. HANNON, M.D., AND WM. S. McCANN, M.D.

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The study of the balance between ketogenic and anti-ketogenic substances in the metabolism, having been put on a quantitative basis by the admirable work of Shaffer,^{1, 2, 3} almost immediately found its application in the treatment of diabetes mellitus. Shaffer found that the ketogenic substances were the fatty acids, and certain of the amino acids of protein. The anti-ketogenic substances were glucose, the glucose yielding amino acids of protein and the glycerol of the fat, which is known to be capable of yielding glucose.⁴ It was found that the complete oxidation of ketogenic substances did not take place unless there were present at least one molecule of anti-ketogenic substance for each molecule of the ketogenic. Shaffer arrived at these conclusions on the basis of *in vitro* experiments, metabolic observations on starving subjects and diabetic patients, including studies of the respiratory exchange.

Woodyatt⁵ has applied Shaffer's data to the calculation of diets for diabetic individuals in whom it is desirable to preserve the proper balance between ketogenic and anti-ketogenic substances. He has converted Shaffer's figures for the molecular proportions into terms of grams of the various foodstuffs. The anti-ketogenic substances are reduced to the term available glucose or G, which equals the sum of glucose available from free carbohydrate, the carbohydrate moiety of the protein molecule, and that which may arise from glycerol of the fats. Thus $G = 0.58 P + 0.1 F + C$, where P=grams of protein, F=grams of fat, and C=grams of carbohydrate.

In calculating the ketogenic substances, expressed as available fatty acids or FA, Woodyatt has not followed Shaffer's figures closely. For instance, 42% of the weight of protein is made up of amino acids which do not yield glucose. Of these only three, leucine, phenylalanine, and tyrosine, are known to be ketogenic, making up only 23% of the total weight of protein. Woodyatt calculates $FA = 0.46 P + 0.9 F$. In view of the unknown behavior of the remaining amino acids of protein it is believed that Woodyatt's estimate is on the safe side.

Woodyatt estimates that the equimolecular ratio of ketogenic to anti-ketogenic substance obtains when

$$\frac{FA}{G} = \frac{1.5}{1} = \frac{0.46 P + 0.9 F}{0.58 P + 0.1 F + C}$$

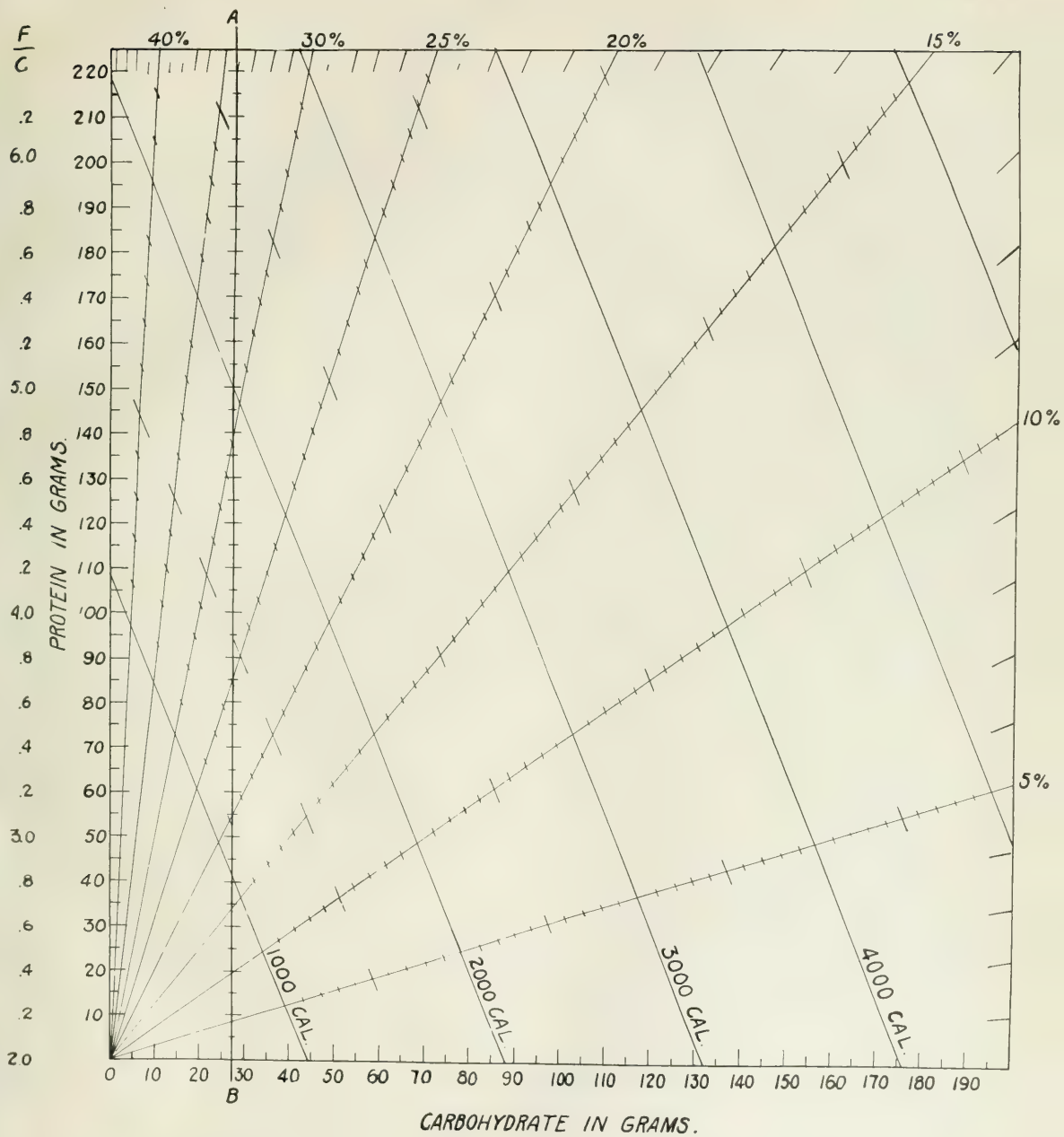
This on being simplified gives the expression

$$F = 2 C + 0.5466 P.$$

In discussing the objects of dietary adjustment in diabetes, Woodyatt has called attention to a fundamental

conception which has been overlooked by practitioners who have followed the methods of treatment of diabetes largely in vogue in the past few years. This is the fact that the tolerance of the patient for glucose cannot be estimated simply from the amount of free carbohydrate in the diet, but that it must be estimated in terms of available glucose from all sources. That since the metabolism goes on uninterruptedly during starvation considerable amounts of glucose may be arising in the organism especially if the protein metabolism is high. He showed in an illustrative case, a patient with severe diabetes in whom the protein metabolism was presumably high (urinary nitrogen figures not given), that the giving of a diet low in protein with considerable fat and free carbohydrate was well borne. This was presumably due to a sparing of body protein by fat, in keeping with the results of Newburgh and Marsh,⁶ though unfortunately exact calculations cannot be made, as there were no data regarding urinary nitrogen or heat production of the patient, except a rough calculation of the requirements based on body weight.

However, the conceptions of diabetes set forth above seemed so rational that it was determined to introduce it in the practice of the medical clinic of the The Johns Hopkins Hospital. Since its introduction very satisfactory results have been obtained. To facilitate the work of preparing the diet prescriptions one of us (R. H.) has prepared the graphic chart, Fig. 1, which has been so useful that we have been led to make it available for general use. All points on the graph bear the relationship between protein, fat and carbohydrate expressed in Woodyatt's equations previously given. The chart is given much additional value by reason of the fact that it provides for the calculation of this relationship over a wide range of levels of protein metabolism. In the plan outlined by Woodyatt⁵ the total caloric requirement of the patient was estimated on the basis of body weight, and an arbitrary figure for protein was used, 1 gram protein per kilogram of body weight. It seemed more rational to estimate the measure of total metabolism in terms of calories per square meter of body surface area, and to consider the protein metabolism in terms of percentage of the total. Thus, on the graph, diagonal lines represent the total calories. Radiating lines from the intersection of the axes represent the percentage of total calories in the form of protein. The ordinates represent grams of protein, the abscissae the grams of carbohydrate which are required to maintain ketogenic-anti-ketogenic balance for any given level of protein metabolism. Thus



if the patient requires 2000 calories per diem, and if it is desired to give 10% of the calories in the form of protein, the point of intersection of the 2000 caloric line and the 10% line locates on the ordinate 49 gms. of protein, and on the abscissa 68 gms. carbohydrate.

Total calories—(calories from protein+carbohydrate) —grams fat.
9.3

To avoid this latter calculation a second graph has been superimposed upon the first. A vertical line AB, parallel to the ordinate axis, is intersected at various points by the radiating percentage lines. For each intersecting point a second ordinate scale gives the ratio of fat to carbohydrate F/C. This is the factor by which the weight of carbohydrate must be multiplied to give the requisite number of grams of fat.

Thus the 10% line intersects the line AB at a point which indicates that F/C is 2.39. Then the number of grams of fat required $=2.39 \times 68 = 162.5$. The prescribed diet would then be protein 49 gm., carbohydrate 68 gm., fat 162.5 gm. This diet would furnish 1920 calories, and would yield a maximum of 113 grams of glucose in the metabolism.

If the minimal protein metabolism for a particular diabetic patient were known (urine N. figures), and if his caloric requirement were properly estimated or measured, it would then be possible to find the minimum carbohydrate and the maximum fat allowable if the patient is to be maintained at the minimal level of nitrogen balance. For instance, a man 30 years old, 170 cm. in height, weighing 60 kg., requires at least 1612 calories per diem. After a period of observation on a diet furnishing 5% of the calories from protein, it is found that his protein metabolism, as shown by the urinary nitrogen excretion, has reached a minimum of 50 grams per diem. Reference to the chart shows that this constitutes 12.6% of his total metabolism. If a diet is to be given which will just cover this minimal protein requirement (50 gm. protein), the quantity of carbohydrate which is prescribed will be 51 gms., and of fat $2.53 \times 51 \text{ gms.} = 129 \text{ gms.}$

It will be found interesting to make the following calculation of two diets each furnishing 2000 calories from the graph. In one case the protein forms 40% of the total energy value. In the other case protein furnishes only 10% of the total energy.

	2000 caloric diets.			Available Glucose
	P	F	C	
40% Protein Cals.	195	125	9	134.6
10% Protein Cals.	49	165	68	112.9

It will be seen that a patient, whose tolerance might be just sufficient to permit the taking of 2000 calories with the smaller amount of protein, would probably

develop hyperglycemia or glycosuria if the same number of calories were given with the higher percentage of protein. The diet containing less protein permits the use of much greater amounts of free carbohydrate.

It is of interest also in this connection to consider the diet of the Eskimo, which has been studied by Krogh and Krogh,⁷ who found that about 44% of the total energy was derived from protein. The graphic chart shows at a glance that in such a dietary very small amounts of free carbohydrate serve to prevent ketogenesis.

It is a matter of common observation, to which attention has been called by Allen and DuBois,⁸ that the level of protein metabolism is almost invariably high in patients with severe diabetes and acidosis. One might well ask, therefore, whether an increase in amount of protein metabolized may not be one of the mechanisms by which the diabetic organism protects itself against undue ketogenesis.

At a recent meeting of the American Society of Biological Chemists, Shaffer made a further report of his studies on antiketogenesis which indicated that at times as much as two molecules of fatty acid can be metabolized completely in the presence of one molecule of antiketogenic substance. Since the 2:1 ratio does not hold in all cases, it has seemed much better to adhere to the formulae constructed on the basis of the 1:1 ratio, as this gives a small margin of safety. The data regarding the molecular proportions of ketogenic and antiketogenic substances in the metabolism of our patients will be given in a subsequent communication.

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THE ALVEOLAR AND BLOOD GAS CHANGES FOLLOWING PNEUMECTOMY

By GEORGE J. HEUER and W. D. W. ANDRUS.

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In some previous experiments¹ it was found that very shortly after the removal of one lung the remaining lung would begin to enlarge, and by this enlargement eventually would fill the entire thoracic cavity. As the lung enlarged, it displaced the heart so that this organ later came into contact with the lateral thoracic wall, and by necropsy examinations it was repeatedly demonstrated that when the heart was in contact with the thoracic wall the enlarged lung completely filled the thorax. A method was, therefore, at hand of following the enlargement of the lung by observing the displacement of the heart with the fluoroscope, and as a result of many examinations it was found that the lung which remained after a total pneumectomy completely filled the thorax in from four to six weeks. The capacity of the lung to undergo this compensatory enlargement seemed very great. Even when not only the entire lung upon one side but the largest of the three lobes upon the other side were removed, the two remaining lobes of one lung, with the exception of the space obliterated by the retraction of the thoracic wall and the elevation of the diaphragm, would enlarge to fill the entire thorax. As to the exact nature of this enlargement we have no knowledge at present, but some work to determine this point is being carried on in the Hunterian Laboratory.

This anatomical change in the remaining lung we have naturally considered a compensatory enlargement—an attempt on the part of the organism to replace the loss of lung tissue. As has been stated, the enlargement begins early, but it requires from four to six weeks to reach its maximum. Yet during this period of four to six weeks the animals apparently do not suffer from the lack of lung substance. They are lively and active; they are not dyspneic. We have thought, therefore, that there must be some immediate functional compensatory mechanism whereby the respiratory needs of the animal are probably assured, until the comparatively late anatomical changes in the remaining lung shall have taken place. The purpose of the present work was to discover whether or not there was such an immediate compensatory mechanism.

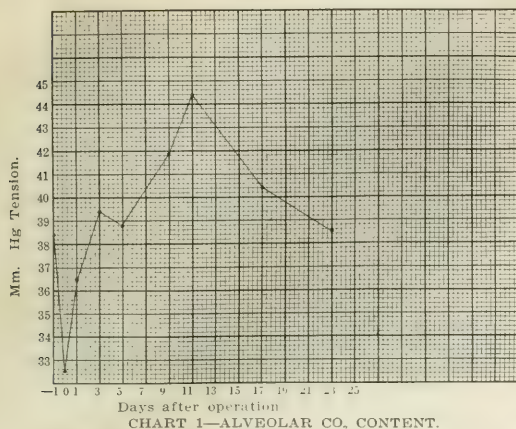
In such a mechanism probably the circulation and

respiration both play a part, the circulation by an increase in the rapidity of blood flow through the remaining lung, the respiration by changes in the alveolar air and blood gases. It is only with the latter—the changes in the alveolar air and blood gases—that this report concerns itself.

METHODS.—In animals, the subjects of these experiments, determinations of (1) the alveolar carbon dioxide content, (2) the alveolar oxygen content, (3) the carbon dioxide content and capacity of the blood plasma, (4) the oxygen content and capacity of the whole blood, (5) the hemoglobin content and red cell count, were made before operation to serve as controls for subsequent similar determinations. The alveolar air was collected by the Plesh² method, the animals rebreathing a certain amount of air from a bag until equilibrium was established between it and the air in the lungs. Samples from the bag were then analyzed in a Haldane apparatus for carbon dioxide and oxygen. The collection and analysis of the blood were made by the method of Van Slyke,^{3,4} all necessary precautions being taken to eliminate contact of the blood with the air or loss of blood gases by diffusion. All samples both of air and blood were obtained after the dogs had been absolutely at rest for at least ten minutes. The observations on the alveolar air and blood gases were made immediately after operation, 24 hours after operation, three and five days after operation, and thereafter at intervals of several days to a week. Controls were made upon animals subjected to operations other than pneumectomy, in order to eliminate the possible effects of operations themselves upon our findings.

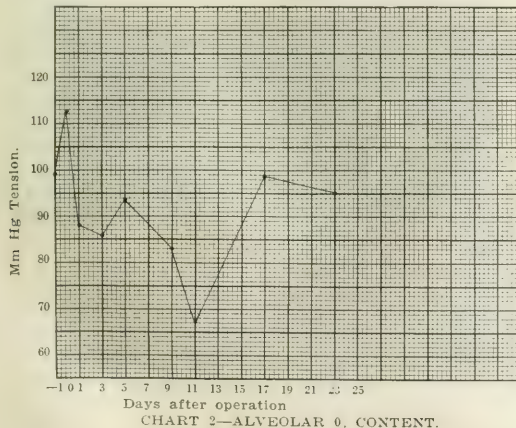
SUMMARY OF RESULTS.—(a) *Effect on Alveolar Carbon Dioxide.* The increased ventilation of the lungs due to anesthesia causes a fall in the alveolar carbon dioxide tension. After operation, however, the alveolar carbon dioxide tension rises, and within 24 hours is about equal to or above the normal value as determined before operation. It continues to rise until about the eleventh day, then gradually falls, reaching normal by about the twenty-fifth day (Curve 1).

FIG. 1.



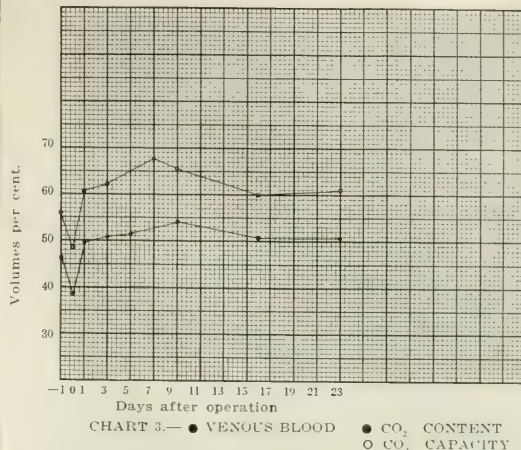
(b) *The Effect on Alveolar Oxygen.* Anesthesia causes a rise of the alveolar oxygen of approximately 2 per cent above the normal value as determined before operation. Following operation the alveolar oxygen shows a marked fall, averaging $3\frac{1}{2}$ per cent, until about the eleventh day, after which it again tends to rise to its normal level. In two animals it reached the pre-operative value in twenty-eight days; in another it had not reached its former level in sixty-six days. The second rise in the alveolar oxygen value occurs coincidentally with the fall in alveolar carbon dioxide tension (Curve 2).

FIG. 2.



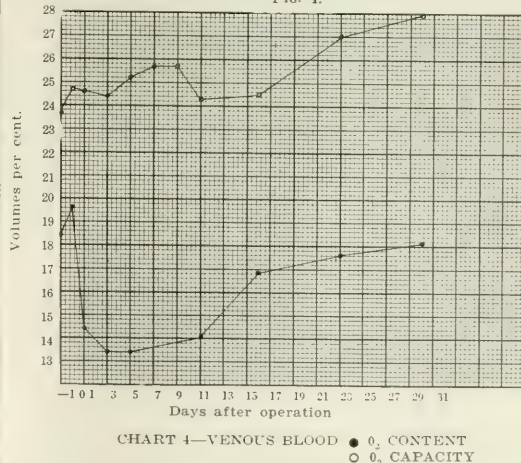
(c) *The Effect on Blood Carbon Dioxide Content and Capacity.* The anesthesia causes an average fall in the carbon dioxide content of the blood of from 8 to 10 volumes per cent. For a period of ten days after operation there occurs a rise of from $4\frac{1}{2}$ to 13 volumes per cent (average, $9\frac{1}{2}$) above the normal value as determined before operation; then a gradual fall to about normal by the twenty-fifth day (Curve 3).

FIG. 3.



(d) *The Effect on the Blood Oxygen Content.* Anesthesia causes a slight rise of about 2 volumes per cent. This is followed within 24 hours after operation by a marked fall of approximately $4\frac{1}{2}$ volumes per cent, in one case by a fall of 11 volumes per cent, or nearly half the total oxygen capacity of the blood. This fall in the blood oxygen content continues approximately up to the eleventh day after operation. In two cases it again rose to normal by the twenty-fifth and the thirtieth days; in one animal it remained at a low level until the twenty-third day (Curve 4).

FIG. 4.

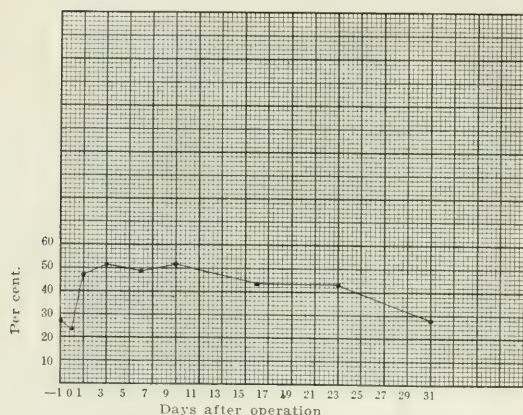


(e) *Effect on Blood Oxygen Capacity.* The effect of anesthesia is in our experience variable, there being either a rise or fall in the blood oxygen capacity. Following operation there is an average rise in the oxygen capacity of the blood of $3\frac{1}{4}$ volumes per cent, this increased value being maintained during the period of our observations

(sixty-six days.) In one animal dying from distemper twenty-eight days after operation, there was a progressive fall in the oxygen capacity (Curve 4).

(f) *The Effect on Oxygen Unsaturation Percentage.* Anesthesia causes a rise in the oxygen unsaturation percentage in two cases, a fall in three cases. Within 24 hours of operation there is a very marked rise of from 9 to 30 per cent, with an average rise of 19.6 per cent. This rise in the oxygen unsaturation percentage continues up to the eighth day, at which time the average rise is 23.4 per cent. Subsequently, there is a fall but not quite to the previous normal value as determined before operation (Curve 5).

FIG. 5.

CHART 5--VENOUS BLOOD. O₂ UNSATURATION PERCENTAGE.

(g) *Observations on the Blood Count.* The day following operation there is an average rise in the red cell count of 666,000. On the third day after operation this rise averages 800,000 above normal, one animal showing an increase of 1,200,000 red cells. This rise in the red cell count corresponds to an increase in hemoglobin of from 15 to 20 per cent. So far as our observations go (extending to the sixty-sixth day in one case) this increase in the red cell count is maintained, two dogs observed on the fifth-third and sixty-sixth days having blood counts of 7,000,000 or more (Curves 6 and 7).

Briefly, then, the effects of pneumectomy are a rise in alveolar carbon dioxide and a fall in alveolar oxygen, these alveolar air changes being associated with a temporary rise in the carbon dioxide content and capacity of the blood, a marked fall in the oxygen content, and a marked rise in the percentage of oxygen unsaturation. Concomitantly, there is a marked rise in the hemoglobin content of the blood and therefore in its oxygen-carrying

FIG. 6.

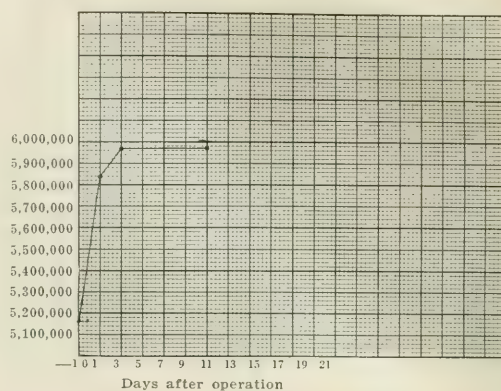


CHART 6--RED BLOOD COUNT.

FIG. 7.

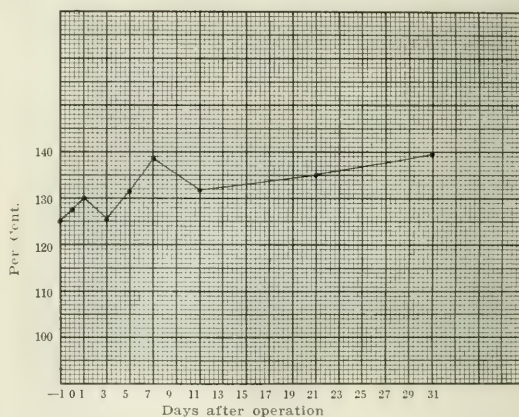


CHART 7--HEMOGLOBIN (Calculated)

capacity, which may be interpreted as a compensatory mechanism. As has been noted, the changes in the alveolar carbon dioxide, alveolar oxygen, carbon dioxide content and capacity of the blood, and percentage of oxygen unsaturation, are only temporary; and approximately within thirty days normal relations are again established. The increased hemoglobin and oxygen-carrying capacity of the blood, however, persisted during our period of observations (sixty-six days). They serve at least in part to supply the respiratory needs of the animal perhaps until complete anatomical changes in the remaining lung have taken place.

PROTOCOL OF EXPERIMENTS

Dog	Date	Day	ALVEOLAR AIR				VENOUS BLOOD						
			CO ₂ Per cent.	CO ₂ Tension	O ₂ Per cent.	O ₂ Tension	CO ₂ Content	CO ₂ Capacity	O ₂ Content	O ₂ Capacity	O ₂ Unsaturation Per cent.	Hemoglobin (calculated)	R.B.C. Count
T ₁	11/ 1/21	—1	5.34	40.6	15.72	119.5	39.8	46.4	14.64	19.03	23.1	103.4	
	11/11/21	+9	5.04	38.3	11.23	85.3	41.4	48.1	16.47	20.95	21.4	112.8	
	11/18/21	16	5.10	38.7	10.43	79.2	52.3	58.6	15.13	20.95	23.0	112.8	
	11/25/21	23	5.39	40.9	11.22	85.2	57.8	62.5	14.82	24.41	39.3	131.9	
	12/ 2/21	31	5.48	41.6	10.23	77.7	51.9	55.7	17.18	24.12	28.7	130.4	
	12/15/21	44	5.38	40.8	11.71	89.0	49.4	53.2	14.50	23.70	38.8	127.5	7,192,000
	1/ 6/22	66	5.21	39.6	13.26	100.8	42.4	48.1	14.87	23.54	36.8	127.2	7,360,000
T ₅ *	11/12/21	—2	4.76	36.1	12.98	91.8	41.6	62.5	13.66	19.00	28.6	102.7	
	11/14/21	0	3.45	26.1	15.60	118.5	35.0	38.7	15.36	19.08	19.5	103.1	
	11/15/21	+1	3.51	26.6	13.50	102.6	35.0	42.4	9.70	15.95	39.2	86.2	
	11/17/21	3	4.74	36.0	12.95	98.3	44.5	48.4	10.25	21.14	51.5	114.3	
	11/19/21	5	5.08	38.6	12.39	94.16	46.4	52.0	7.90	16.40	57.9	88.7	
	11/22/21	8					50.2	57.6	5.46	17.29	56.8	93.5	
	11/25/21	11	5.61	42.5	10.00	76.0	43.6	48.5	9.75	16.72	41.7	90.4	
	11/30/21	16	5.07	38.5	11.83	89.9	43.3	50.5	7.80	16.38	52.5	88.5	
	12/ 5/21	23	4.80	36.4	11.99	91.1	42.3	47.5	6.44	14.53	55.7	78.5	
T ₆	11/14/21	—1	5.07	38.5	12.80	97.3	47.3	55.9	15.81	23.12	31.7	125.0	
	11/15/21	0	3.60	27.4	16.01	121.7	38.8	49.2	17.74	22.42	20.7	121.2	
	11/16/21	1	4.80	36.5	12.20	92.7	57.7	72.0	9.26	23.24	60.15	125.6	
	11/18/21	3	5.39	40.9	11.00	83.6	52.0	61.6	12.95	21.95	41.0	118.6	
	11/21/21	6	5.29	40.2	11.10	84.3	59.6	67.2	11.91	24.45	51.3	132.1	
	11/22/21	7	6.24	47.4	11.08	84.1	59.2	79.0	14.23	25.59	44.4	138.2	
	11/26/21	11	6.12	46.5	8.38	63.7	60.1	67.5	18.34	24.89	26.3	134.8	
	12/ 1/21	18	5.80	44.1	12.00	91.2	52.5	58.5	14.29	22.86	37.2	123.9	
	12/ 8/21	25	4.97	37.7	12.28	93.3	50.0	59.5	15.02	22.97	34.6	124.2	
	12/15/21	32	5.62	43.3	11.32	87.16	51.6	56.5	16.42	23.69	31.9	128.0	
	1/ 3/22	53	5.12	38.9	12.37	94.0	47.1	53.8	14.21	24.26	40.6	131.1	7,000,000

* Died of distemper on the 28th day after operation

PROTOCOL OF EXPERIMENTS—Continued.

Dog	Date	Day	ALVEOLAR AIR				VENOUS BLOOD						
			CO ₂ Per cent.	CO ₂ Tension	O ₂ Per cent.	O ₂ Tension	CO ₂ Content	CO ₂ Capacity	O ₂ Content	O ₂ Capacity	O ₂ Unsaturation Per cent.	Hemoglobin (calculated)	R.B.C. Count
T ₇	11/26/21	—1	5.25	39.9	14.05	106.8	52.0	56.5	16.91	23.72	28.8	128.2	5,296,000
	11/27/21	0	4.20	31.9	16.08	112.9	49.5	55.2	19.02	22.44	15.2	121.3	
	11/28/21	1	5.23	39.6	11.31	86.0	52.4	59.0	15.86	25.52	37.9	137.9	5,872,000
	11/30/21	3	5.42	41.2	12.22	92.9	61.3	65.3	14.29	25.21	43.4	136.3	6,480,000
	12/ 5/21	8					51.0	61.0	16.22	27.61	41.3	149.2	6,096,000
T ₈	12/ 3/21	—1	5.02	38.2	11.14	84.7	48.1	59.5	19.16	24.27	21.1	131.1	5,392,000
	12/ 4/21	0	4.94	37.5	12.94	98.3	33.8	40.0	20.11	25.51	26.7	137.4	5,700,000
	12/ 5/21	1	5.23	39.7	9.60	73.0	44.3	52.3	13.10	26.25	50.1	141.9	5,970,000
	12/ 7/21	3	5.35	40.7	9.29	70.6	52.4	60.0	8.42	25.69	67.2	138.8	5,816,000
	12/10/21	6	4.89	37.1	13.36	101.5	49.5	59.5	12.87	23.61	45.5	127.4	4,888,000
	12/13/21	9	5.26	39.5	10.89	52.8	46.2	53.8	10.26	22.06	50.3	119.6	5,328,000
	12/16/21	12	5.37	41.0	10.95	83.2	51.9	55.7	10.12	25.21	59.8	136.2	5,912,000
	1/ 3/22	28	4.96	38.1	12.00	93.2	47.1	52.8	12.38	25.35	46.1	137.0	5,872,000
T ₁₀	12/11/21	—1	4.87	37.0	12.42	94.4	45.8	55.3	18.00	25.39	29.1	137.2	4,816,000
	12/12/21	0	5.22	39.7	13.36	101.5	51.5	55.7	18.39	28.95	36.1	156.4	4,896,000
	12/12/21	6 hrs.P.O.	5.52	41.9	10.95	83.2	51.9	59.5	15.53	24.99	37.9	135.1	4,944,000
	12/13/21	1	5.30	40.3	11.44	86.9	46.5	55.7	13.99	25.76	45.7	142.7	5,560,000

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POSTERIOR RESECTION OF THE RECTUM AND RECTOSIGMOID (KRASKE OR MODIFIED) UNDER REGIONAL ANESTHESIA*

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Apart from early diagnosis and the choice of methods in dealing with cancer of the rectum and rectosigmoid, one of the most interesting problems connected with the surgery of that portion of the large intestine is the choice

of the method of anesthesia, especially if the operability is raised, as has been the tendency for the past few years.

Among the patients suffering from the disease are those with chronic conditions of the urinary, respiratory, or circulatory systems, which make them poor operative risks under ordinary circumstances. Obesity is apt to

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increase the mortality, or at least to cast a heavy shadow on the operative prognosis. The colostomy itself, in the case of very obese persons, is attended by considerable risk.

Nephritis is one of the important causes of operative mortality. It is usually of an acute type, superimposed on a chronic process. Many patients die some weeks after the operation from nephritis, cardiovascular disease, and so forth, which they had at the time of the operation. Infection (general peritonitis, pelvic cellulitis), obstruction and exhaustion are, according to the present study, the most frequent causes of death.

Although the anesthetic agent used for long and traumatizing operations such as the Kraske or any other type of posterior resection of the rectum is seldom mentioned in the literature, the presumption is that it has almost always been general anesthesia by ether inhalation. The morbid influence of ether on lungs, kidneys, and arteries with pathologic lesions is too well known to be deserving of more than mere simple mention in this paper. The fatal blow given by the lipid solvent to the progressively exhausted heart laboring under fatty degeneration needs no discussion. In apparently normal persons, the unfavorable influence of ether on the kidneys, lungs, and gastro-intestinal tract is most apparent in the early days following operation; and the patient recovers generally without any complication attributed to the anesthetic. Albuminuria, glycosuria, leucopenia, glycemia and acidosis, all clear up within a relatively short time. But occasionally a condition of exhaustion exists at the time of the operation, a condition chiefly due to anesthesia or the inability of the patient to adapt himself to ether inhalation. Some patients recover from this condition; but others find their power of resistance gradually decreasing. The natural reaction to infection is thus greatly diminished. Cellular life is weakened in the central nervous system which loses control over the vital functions of the economy. The kidneys at times find themselves helpless to deal with the abundant waste material coming directly from the operative wound, and the material which overflows or leaks from the defective liver.

Such disturbances may be avoided or considerably lessened by the use of regional anesthesia, which does not, as a rule, affect the general condition of the patient. But it must be clearly understood that the block method is not meant to improve chronic conditions which are present at the time of the operation, nor to prevent the development of acute disease started just before the operation. Regional anesthesia does not increase the resistance of the patient, but it reduces to a minimum an unfavorable operative prognosis by not affecting the vital functions of the main organs of the body. Regional anesthesia does not prevent pulmonary embolism, but it takes away the danger of pulmonary complications, since it does not interfere with the respiratory organs. Edema

of the lungs, pulmonary congestion, or pneumonia will not set in as a postoperative complication if the usual elementary precautions are taken. Since regional anesthesia does not affect the gastro-intestinal tract, there is no postoperative nausea and vomiting, no acute distension of the bowels with consequent paresis, and no strain on the abdominal cavity and its contents immediately after the operation. Postoperative paralytic ileus is prevented more effectually by the normal peristaltic movements of undisturbed bowels. Tendency to bleed is also greatly reduced and convalescence rendered shorter.

In considering the advantages and the bright postoperative clinical picture shown by the majority of patients who were considered very poor surgical risks before the operation, regional anesthesia has unquestionably strong claims against ether anesthesia or any other form of inhalation narcosis, which occasionally results in serious complications. Regional anesthesia is the method preferred for posterior resection of the carcinomatous rectum and rectosigmoid, whatever be the condition of the patient. In this type of case, it can be said without error that all patients are amenable to the method; but it may be well to emphasize a few points which are occasionally, if not always, overlooked, especially if regional anesthesia is only applied to particularly poor surgical risks.

In trying to increase the percentage of operability, the question of local conditions of the disease and of metastasis is generally solved by exploration at the time colostomy is performed. How far the radical cure is indicated, and the type of operation best suited to the particular case, are matters I shall not discuss, although the choice of operation is a significant factor in the final settlement of the prognosis *quoad vitam* in cases of carcinomatous rectum and rectosigmoid. After the operation has been decided on, two questions remain to be answered: Will the patient stand the operation? Will he stand the anesthesia needed for the operation added to the operation itself? The answers depend on the condition of the main organs and their surgical resistance, measured in terms of their progressive alteration from the time of the colostomy, the coefficient risk being added to the operative prognosis by the personal equation of the surgeon. The risk due to the type of anesthesia has too long been overlooked; and it is now time to consider the opportunity of using regional anesthesia exclusively for the Kraske or any other types of posterior resection of the rectum and rectosigmoid.

The preconceived idea of the absolute safety of regional anesthesia is apt to influence the operability favorably, although it cannot be expected to alter the pathologic conditions existing at the time of exploration. All patients who seem to be good operative risks *quoad* the lesion may otherwise be very poor surgical risks; and, in raising the operability, one should be cautioned against those cases that are on the borderline. In considering

the advisability of a radical cure, the actual resistance of the patient after the colostomy should be the chief guide. Some patients continue to decline after the colostomy, and can scarcely pull through that first-stage operation, especially when it has been performed under ether anesthesia. Progressive dehydration cannot be avoided, and from fifteen to twenty days after, which is the usual time for the second stage operation, these patients are weaker than ever. Whatever anesthesia is then used, they will seldom come out of that condition of progressive exhaustion which silently carries them across the borderline into a state of coma which starts during the operation and ends with life on the second or third day. In those desperate cases regional anesthesia only permits the surgeon to risk the colostomy, and the prognosis of the second-stage posterior resection is greatly improved.

A study of thirty-three cases in which operation was performed in the Mayo Clinic from October, 1920 to June, 1921, inclusively, brings out points which tend to prove the innocuousness of the method of regional anesthesia:

1. Previous diseases do not seem to influence the operative risk, provided no acute process prevails at the time of the operation. Patients having a previous history of diphtheria, pneumonia, grippe, pleurisy, scarlatina, measles, chicken pox, acute Bright's disease, and so forth, whether contracted recently or several years before, have made uneventful recoveries from operations performed by means of regional anesthesia.

2. Chronic lesions of the heart, with or without compensation, are not conditions which contra-indicate the use of the regional method, provided the resistance of the patient seems fair. Patients with enlarged hearts (1.5 to 2 cm. to the left) with extrasystoles, associated with a systolic blood pressure of 165 mm., diastolic 96 mm. and deficient kidneys; patients presenting mitral incompetence and aortic stenosis, with a systolic blood pressure of 140 mm., diastolic 90 mm., and pulse 116, have left the hospital in from ten to twenty-two days following the posterior resection of the anus, anal canal, rectum, and lower sigmoid.

3. Pulmonary tuberculosis does not seem to influence the operative prognosis. An operation was performed under paravertebral and sacral block on a woman, aged thirty-six years, who gave a history as follows: She had had four pregnancies, two of which were extra-uterine, one rupturing. One pregnancy ended in a miscarriage at three months. She had had "anemia" twelve years before; pneumonia, in childhood, and eight and two years before; typhoid fever with peritonitis twenty-two years before; and active tuberculosis ten years before. Her sputum was positive May 4, 1921, when a left inguinal colostomy was performed under regional anesthesia. One month later the anal canal, entire rectum, and 25 cm. of the sigmoid were excised. The growth was in the rectosigmoid, extending down into the rectum. A loop of the

sigmoid and one of the small intestine had dropped down and become adherent to the growth. The small intestine was separated, but the peritoneal coat, where it was adherent, was removed. A loop of the sigmoid about 10 cm. above the growth, which had become adherent to the growth, was removed with it. The glands and fat which were involved and adherent to the promontory and sacrum were removed. The upper end of the sigmoid was turned in twice and fastened to the peritoneal wound. The posterior wall of the uterus was drawn into the cavity to help fill the large peritoneal defect.

The extent and type of the operation performed in this case is a real test of the method of regional anesthesia. No particular care was taken to avoid or lessen the usual manipulations generally adopted under ether anesthesia. The patient made an uneventful recovery and left the hospital on the twentieth day after the operation.

5. Diabetes, at least mild and chronic, easily controlled by dietary measures, has to all appearances no influence on the operative prognosis if regional anesthesia is used. A woman, aged seventy-two years, was operated on for carcinoma of the rectum April 1, 1921, while suffering from mild chronic diabetes. Her urine output was 800 c.c. in twenty-four hours. The urinalysis showed specific gravity 1.016; reaction, acid; sugar, a trace; and pus cells, 1 to 6. The patient left the hospital twenty days after the operation with a clean postoperative record.

6. Operation may also be safely performed in renal conditions of a chronic type. A woman, aged forty-seven years, had an average urine output of 500 c.c. in twenty-four hours, the urinalysis showing specific gravity 1.029; reaction, acid; albumin 1; pus cells 3; and a combined phenolsulphonaphthalein return of 35 per cent. She gave a previous history of having had diphtheria, scarlatina, pneumonia, grippe, and pleurisy, and at the time of the operation was suffering from mitral incompetence and aortic stenosis. A posterior excision was made of the anus, anal canal, rectum, and lower sigmoid. She passed an average of 650 c.c. of urine every day following the operation. She recovered uneventfully, and left the hospital ten days after the operation.

PREPARATION OF PATIENTS FOR REGIONAL ANESTHESIA

For the one-stage Kraske operation, the patient receives a preliminary hypodermic injection of morphin 1/6 gr. and scopolamin 1/300 gr., one hour before anesthesia is begun, and a second dose of the same strength immediately after the completion of the anesthesia.

For the colostomy in the two-stage operation, one hypodermic injection of the narcotics is, as a rule, sufficient to dull the mentality of the patient and dismiss any apprehension due to consciousness at the time of the operation, provided it is given one hour before the anesthesia, and the colostomy is performed ten minutes after the completion of the anesthesia. In very nervous

patients, if the first dose does not seem to have produced the desired effect, a second dose is given at the time of the anesthesia.

The patient is prepared for the second stage Kraske in the same manner as for the one stage. Individual cases should be considered in administering two doses of the combined narcotics. If during the manipulations for inducing anesthesia, the attitude of the patient reveals sufficient psychic control there is no reason for giving the second dose; it should be reserved for alleviating postoperative pain.

The use of morphin and scopolamin in such weak doses is not meant to produce, and in fact does not produce, a twilight sleep nor a semiwaking condition during which the surgeon loses control of his patient, but simply to enable the patient to feel more comfortable in that rather awkward position he must assume during the operation.

ANESTHESIA FOR THE COLOSTOMY

For the left rectus colostomy, two procedures are available, the abdominal field block, and the paravertebral block.

The abdominal field block is the procedure usually employed because it is easily and quickly accomplished, and requires no special long and delicate training. It affords almost complete relaxation of the abdominal muscles and perfect anesthesia of the parietal peritoneum within the blocked area. If gentleness is used, the pelvic organs, as well as the liver, may be explored, provided the incision is long enough to allow the hand to steal into the abdominal cavity without force. This is most easily done after the lips of the wound have been clamped and raised. In the majority of cases, there is no sharp pain during gentle exploration, but only an abdominal sensation referred to the epigastrium, such as that due to intestinal colic. The sensation ceases with the exploration, which ordinarily is of very short duration. Some patients require just enough ether or gas to make them lose consciousness during this stage of the operation. If the excitation period is reached, exploration becomes impossible, owing to lack of relaxation which can only be obtained by bringing the patient to the surgical stage of general anesthesia; and in such case he loses the benefit of the regional anesthesia. It is, therefore, wise to insist on the necessity of a judicious use of ether or gas during exploration.

The average patient, well prepared by the preliminary narcotics, and familiar with the requirements of regional anesthesia, coöperates willingly and, if gentleness is used, the colostomy is painlessly performed. If the patient is obese, or if the mesocolon is short, a few whiffs of a general anesthetic contributes greatly to establish favorable conditions. This combined method is safer than any general anesthetic alone.

The patient lies in the recumbent dorsal position, and the operative field is prepared as for any surgical opera-

tion; one coat of tincture of iodine is sufficient for the purposes of regional anesthesia. Intradermal wheals are raised with a fine needle along the left costal margin, from the xiphisternum to the tip of the eleventh rib, and from that point to the iliac crest, (Fig. 1). A fine needle of convenient length (8 to 10 cm.) is then passed through each of these wheals in succession and deep injections made within the muscle layers of the abdominal wall, followed by subcutaneous injections which join all the wheals, except the first to the last. All the injections along the costal margin are made in a plane perpendicular to the surface of the skin and passing through the wheals; those from the costal margin to the iliac crest are made in a similar manner. Two walls of anesthesia, meeting at about the level of the tip of the eleventh rib, are thus created, cutting off the nerve supply of half the abdominal wall. The anesthetic is a 0.5 per cent solution of neocain* or procain, containing 15 minims of adrenalin solution 1:1000 per 100 c.c. The quantity injected varies from 100 to 200 c.c., according to the weight of the patient.

The paravertebral block is more difficult and, in order to give it successfully, fairly good training is required; but it affords a wider field of anesthesia and greater facility in handling the bowel, in case the mesocolon is short. It is customary to inject from the eighth dorsal to the third lumbar nerve on the left side with from 5 to 6 c.c. of a 1 per cent. neocain or procain solution at each nerve. Paravertebral block thus performed does not abolish the abdominal sensation present during exploration, but it lessens it to a certain extent. In the majority of cases, the colostomy is performed painlessly without the aid of ether or gas. If regional anesthesia is to be induced by the average surgeon, the abdominal field block, according to my experience, should be given preference.

ANESTHESIA FOR THE KRASKE OR ANY MODIFIED POSTERIOR RESECTION

One hour after the preliminary hypodermic injection of morphin and scopolamin has been given, and twenty to thirty minutes before the hour fixed for the operation, the patient is gently wheeled into the operating room or into the special room which, in a few institutions, is specially equipped for inducing regional anesthesia. He is placed flat on his stomach and a cushion slipped under his hips to raise the sacral region and render the landmarks more accessible. The operative field is prepared in the usual manner, exposing the whole lumbosacral region, from the first lumbar vertebra to the tip of the coccyx. One hundred and fifty cubic centimeters of a 1 per cent. procain solution containing 15 minims of adrenalin solution 1:1000 is placed in a cup at hand, and the special regional anesthesia syringe with one set

* Neocain is a French product made by Corbière and Lionnet, Paris, France, put up in sterile capsules of 0.5 gm. each. It is readily soluble in saline solution and of very low toxicity.

of needles of different dimensions tested as to efficiency before starting the manipulations.

The best results are obtained by combining the caudal, transsacral, and paravertebral block of the last three lumbar nerves (Fig. 2), starting with the caudal block, then injecting the sacral nerves, and finishing with the paravertebral block. Whatever be the level of the growth, one and the same procedure should be applied, although anorectal and rectal lesions require a smaller anesthetic field. It is always preferable to prepare the patient in such a way that no subsequent injections will be necessary, if the surgical manipulations are carried beyond the contemplated area. For instance, an error of diagnosis may be followed by the excision of many centimeters of bowel through the posterior route, in order to reach the growth situated high in the sigmoid colon. When the lesion lies in the rectosigmoid juncture, the procedure described here gives absolute anesthesia throughout the operation. It is, therefore, advisable, I insist, not to modify the procedure according to the height of the lesion, but to use it integrally in all cases.

1. *Caudal or epidural block.*—The sacral hiatus is defined by a depression which is felt in the middle line, at about the juncture of the coccyx with the sacrum, and bounded by the sacral cornua on each side and the fourth sacral spinous process on the middle line a little higher up. These three prominences, palpable in the majority of cases, form the angles of a triangular surface at the middle point of which the needle is introduced with ease and success. The spinal puncture needle, with its stylet in and its bevel turned upward, is introduced at this point, in a direction making an angle of about 45 degrees with the surface of the skin. After piercing the sacrococcygeal membrane which, like a screen stretched across the sacral hiatus, closes the lower extremity of the sacral canal, the point of the needle strikes the anterior wall of the canal. It is then withdrawn 1 or 2 mm. and the hub of the needle swung downward toward the gluteal cleft, reducing the angle of 45 degrees to about 20 degrees. The needle is advanced gently and gradually into the sacral canal, always keeping along the middle line, until about 6 cm. of its length have disappeared. If the point of the needle impinges on the posterior wall of the canal and is stopped a short distance from its point of entrance, it is ordinarily released by the application of pressure on the sacrococcygeal membrane with the left forefinger placed on the needle at the site of puncture. If this device is not successful the needle has to be withdrawn and reintroduced a little higher. When the needle has been correctly introduced, its stylet is withdrawn and time is allowed to make sure that no blood or cerebrospinal fluid comes out. In such case the needle is drawn back a few millimeters until the flow ceases and the syringe, filled with a 1 per cent. solution, is connected with the needle. It is a very good practice to aspirate before injecting fluid, so as to feel quite certain that

no intraspinal nor intravenous injection is actually made. Abnormal extension of the dural sac below the second sacral vertebra is exceptional, but its existence cannot be denied. Injection should be very slow, owing to possible after-effects due to rapid absorption by the structures contained in the sacral canal. A total amount of 30 c.c. of the anesthetic solution thus injected into the sacral canal establishes favorable conditions for the transsacral block.

2. *Transsacral block.*—This procedure consists in injecting the sacral nerves individually by introducing the needle through the posterior sacral foramina, which can be reached with some precision only by taking accurate superficial landmarks. These are the posterior superior spine of the ilium and the sacral cornua. A wheal is raised about 1 cm. medial to and below the said iliac spine and another wheal is placed just above the sacral cornu on the same side. The distance between them is divided into three parts by two other intermediate wheals. The second, third, fourth, and fifth sacral foramina are thus easily and accurately defined. The first sacral foramen is found by raising a wheal about 2.5 cm. above that which marks the second sacral foramen, following the same general direction. The thickness of the soft tissues overlying the sacrum being much greater in the upper portion of the bone, needles of different lengths are used according to the height of the puncture. It is most convenient to use a needle 5 cm. long for the last three foramina, one 8 cm. long for injecting into the second sacral foramen, and one 10 cm. long for the first foramen which is very deeply situated (Figs. 3 and 4). It is customary to begin with the second foramen, which seems to be easier to locate than the first and which helps considerably in locating the others. The needle is introduced perpendicularly to the surface of the skin and gently advanced toward the posterior aspect of the sacrum until its point touches the bone. A little practice ordinarily gives an almost accurate tactile sense of the rich fibrous structures overlying the foramen and spreading out, so to speak, in its immediate neighborhood. The direction of the needle is slightly changed if the foramen is not reached by a first puncture; but gentleness should be used in approaching the bone so as not to hurt the patient, traumatize the region by repeated punctures, and damage the point of the needle, which bends to a hook and tears through the tissues while it is withdrawn. This faulty technic of most beginners can be improved rapidly by a short training on the cadaver, especially if every effort is made to visualize the framework through the soft overlying structures. After losing contact of the posterior aspect of the sacrum, the needle passes through the posterior sacral foramen and, according to the foramen, is introduced more or less deeply, because of the uneven thickness of the bone. For the same reason the quantity of solution injected varies with the foramen. It is customary to inject 6 c.c. of the solution in the first

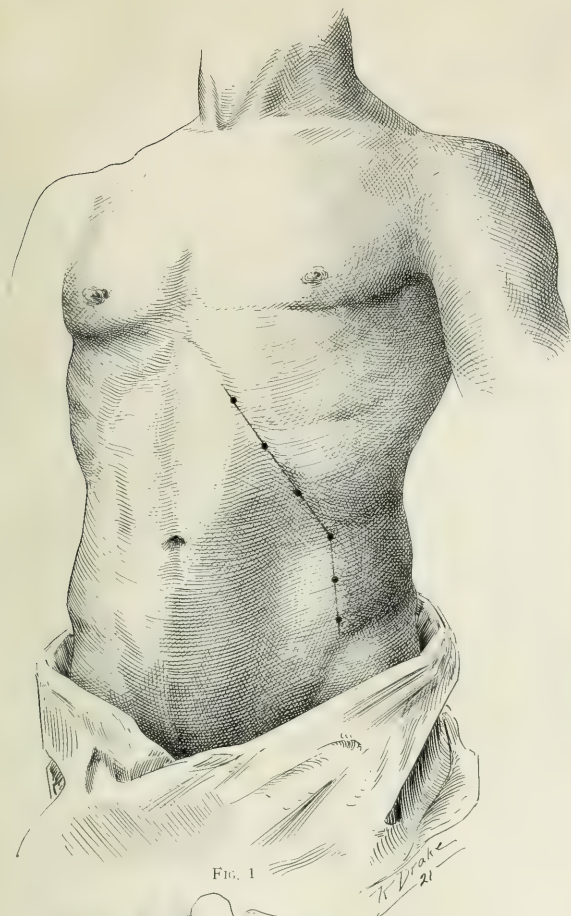


FIG. 1

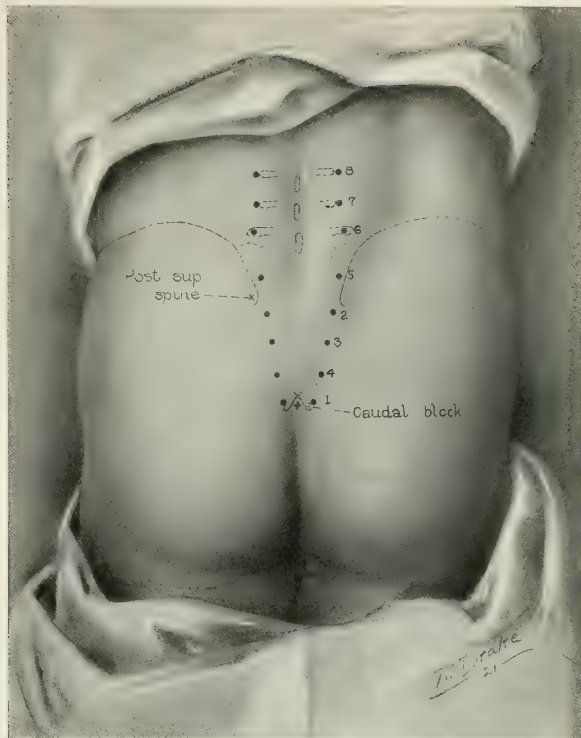


FIG. 2



FIG. 3

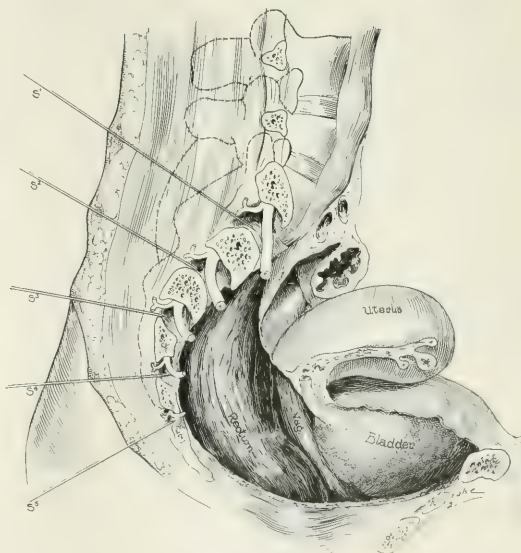


FIG. 4

foramen and to reduce by 1 c.c. each time the quantity injected into each subsequent foramen. Starting from the second foramen injection is therefore made of 5, 4, 3, and 2 c.c. respectively, in descending order of foramen, thus injecting 40 c.c. of the solution, which, added to the 30 c.c. used for the caudal block, makes a total of 70 c.c. for the sacral block.

3. *Paravertebral block.*—After inducing the sacral block, as described, bilateral injections of the last three lumbar nerves are made as follows: Wheals are raised 3.5 to 4 cm. from the middle line of the spine, according to the weight of the patient, opposite the upper edge of the spinous process of the third, fourth, and fifth lumbar vertebræ (Fig. 2). A needle 12 cm. long is introduced through each of these wheals in succession and advanced in a direction perpendicular to the skin surface until contact is taken with the transverse process of the vertebra. The needle is then withdrawn a little, for the purpose of changing its direction, and reintroduced toward the spine, making an angle of about 20 degrees with the middle plane on the body. The needle passes above the transverse process and is stopped at 2.5 to 3 cm. after it has passed the bone, and 8 to 10 c.c. of the solution is injected while the needle is slightly moved to and fro. Efforts should be made not to hit the nerves, but to deposit the solution in their immediate vicinity. The same rule applies to the sacral block. In order to reach the fifth lumbar nerve the needle is passed below the transverse process of the fifth lumbar vertebra. The aspiration test for blood should be renewed several times during each injection, thus making sure that the point of the needle does not lie within the lumen of a blood vessel. Not more than the exact quantity of the solution should be taken in the syringe each time, so as not to exceed the total amount of 150 c.c. of the 1 per cent. solution prepared beforehand. The injection of greater quantities is likely to produce toxic symptoms. Procain is ten times less toxic than cocain, but still its toxicity must be remembered, and likewise the possible presence of impurities in the commercial drug. Toxic symptoms are exceptional; but if they should appear, a subcutaneous injection of spartein sulphate 0.05 gm., caffein 0.25 gm., and strychnin sulphate 0.001 gm. improves the condition. No such mishap is expected from the method itself which is the safest now known; but attention should be called to the fact that it may occur through faulty technic, and the anesthetist should know what to do in such cases.

After making the last injection, the sensibility of the anus and perineum is tested by clamping the region. Complete relaxation of the anal sphincter is a proof that the entire rectum, bladder, and prostate have been anesthetized, although there is no way of testing beforehand the sensibility of the parietal peritoneum in connection with the mesosigmoid, and the pelvic organs in women.

CONCLUSIONS

1. The choice of the method of anesthesia is one of the most interesting problems connected with the surgery of the rectum and rectosigmoid.

2. Most of the postoperative complications may be attributed to inhalation narcosis, especially ether, and can be avoided or considerably lessened by the use of regional anesthesia.

3. Regional anesthesia does not increase the resistance of the patient, but leaves the vital functions of the body in the same condition.

4. The anesthesia does not prevent pulmonary embolism, but excludes the danger of pulmonary postoperative complications, provided no acute condition exists at the time of the operation.

5. The anesthesia has no ill-effects on the gastrointestinal tract; thus the possibility of paralytic ileus and the tendency toward hemorrhage are reduced.

6. All patients are amenable to the method, but one should be cautioned against borderline cases.

7. Previous conditions, such as chronic lesions of the heart, with or without compensation, high blood pressure, pulmonary tuberculosis, diabetes, at least mild and chronic, easily controlled by dietary measures, and chronic renal conditions do not seem to interfere with the operative prognosis.

8. The use of morphin and scopolamin controls the psychic state of the patient and greatly contributes in establishing favorable conditions during the operation; but the stage of twilight sleep must not be reached.

9. With the abdominal field block procedure, colostomy is performed painlessly, provided the patient is not too obese and the mesocolon is not too short. Exploration is possible in the majority of cases, if gentleness is used.

10. The sacral block, consisting of the caudal or epidural and trans-sacral block, added to the paravertebral block of the last three lumbar nerves on both sides, constitutes the method of choice for the posterior resection of the carcinomatous rectum and rectosigmoid.

11. The administration of the anesthesia is not difficult, but it requires practice and patience, irrespective of the gentleness which must always be used in handling conscious patients.

12. If the anesthetic does not give complete anesthesia throughout the operation, the administration of a first stage ether anesthesia during the deep manipulations constitutes a combined method much safer than general narcosis alone.

LEGENDS

Fig. 1.—Abdominal field block for Colostomy, resulting in anesthesia of left half of abdominal wall.

Fig. 2.—Regional anesthesia for posterior resection of rectum and rectosigmoid.

+ Site of puncture for caudal or epidural block.

1, 4, 3, 2, 5. Sites of puncture for transsacral block.

6, 7, 8. Sites of puncture for paravertebral lumbar block.

Fig. 3.—Oblique longitudinal section of the pelvis showing the right sacral foramina, the entire sacrum being preserved. This is intended to show the variable thickness of the soft tissues overlying the sacrum and the different directions of the needle.

Fig. 4.—Oblique longitudinal section of the pelvis passing through the right sacral foramina, showing the progressively decreasing thickness of the sacrum from top to bottom, a condition which commands the use of variable quantities of anesthetic solution according to the site of injection.

THE USE OF THE BONE GRAFT IN THE TREATMENT OF POTT'S DISEASE.

By WILLIAM S. BAER, M.D.

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The ten years which have now elapsed since Albee first called attention to the use of the bone graft as an aid in the cure of tuberculosis of the spine, furnish a period of time sufficient to allow us to draw some conclusions as to the value of the procedure. With this aim in view I have collected the cases of tuberculosis of the spine treated by this method by myself or my associate, Dr. Bennett, and our assistants.* The cases to be reported are fifty in number and in each one the bone graft operation was done prior to December 31, 1919. Thus two years at least, and in many cases more, have elapsed since the operation, so that a careful analytical study of the series will throw some light upon the efficacy of the operation and the indications for its employment.

We have arbitrarily divided our cases into three groups, according to the results obtained.

FIRST: Good results. In this class there are

- No symptoms or signs of active tuberculosis in the vertebral column.
- No support is necessary.
- There is no kyphosis present or no increase in the preoperative kyphosis.

SECOND: Fair results. In this class there are

- No symptoms or signs of active tuberculosis.
- No brace or support has to be used at the present time.
- A post-operative increase of the kyphos has occurred.

THIRD: Poor results. Patients in whom the process is still active; those in whom support is still necessary, or those who have died.

Again, the cases have naturally divided themselves into three groups, according to age at the time of operation.

- Up to the age of six years, the period of infancy.
- From six to sixteen years of age, the period of greatest growth.

- From sixteen years upwards,—the period when growth for the most part has ceased.

This means that in Class *a*—up to six years of age—our results have been as follows:

Good	— 7. %
Fair	— 21.5%
Poor	— 71.5%

In Class *b*—six years to sixteen years:

Good	— 33-1/3%
Poor	— 66-2/3%

In Class *c*—sixteen years and upward:

Good	— 90%
Poor	— 10%

These figures go to show that if the operative procedure is instituted before the age of six years the ultimate result is apt to be poor, while between the age of six to sixteen years it is somewhat better. If, however, we include in class *a* under good results the cases in which all symptoms have ceased, but where the kyphos has increased, the relative comparison is a good result in class *a* of 28.5% against a good result in class *b* of 33-1/3%, or, almost the same. According to these figures, then, operative procedures in the ages of infancy and of growth, have been successful in less than one-third of the cases, even if we are so lenient as to include those cases in which the kyphosis has been increased.

On the other hand, in class *c*, of the patients operated upon after the age of sixteen, ninety per cent have given a good result. This would tend to show that in adult cases in which growth of the body has ceased, and in which the human body has concentrated its efforts on repair, the addition of a bone graft has given excellent results. The rationale of this improvement may be that the bone graft acts as an intra-corporeal support so that traumatism is better resisted; or that the blood supply between the various vertebral bodies is increased and changed by the new medium placed along the spinous processes.

* I am greatly indebted to Dr. Winthrop Phelps for his work in correlating these cases.

Localization of the disease:

For the cervical region the poor results were 100%.
 For the mid-dorsal and lower dorsal region there were good results in 39% and poor results in 61%.
 For the dorso-lumbar and lumbar region there were good results in 73% and poor results in 23%.

Those regions of the spine in which the vertebral body is the largest and hence relatively more resistant to necrosis have given better results. Contrary to what would naturally be supposed, the amount of lessened mobility of the spine, owing to anatomical conditions, as in the mid-dorsal and dorsal region, does not seem to have had any favorable influence on the progress of the case toward recovery.

The effect of the presence of a kyphosis on the ultimate result of the operation: In the cases in which there was no *kyphosis* at the time of operation the result of the operation was 100% recovery. In those in which there was a *slight kyphosis* at the time of the operation, the result was a cure in 50% of the cases. In the cases in which there was a *moderate kyphosis* there was a cure in 50% of the cases. In those in which there was a *marked kyphosis* there was a cure in only 25% and a failure in 75%. In other words, the greater the kyphosis at the time of operation the less the chances of a cure. There are several factors which probably afford an explanation of these results.

Tuberculosis starting after the age of growth is far less likely to produce a kyphosis than when the human body is in the growing stage. Most of the patients who have little or no kyphosis are adults. As we have already mentioned, in adult cases a cure is effected in 90%.

When a kyphosis is large, the bone graft is usually weakened in making it conform to the curve of the kyphosis; indeed, under these circumstances it has often been broken. The graft may break at the time of the operative procedure, or it has been known to hold for as long as two years after it has been embedded and then break. It has further been noted that when a graft has broken, the acute symptoms, which had been overcome by the operation, have reappeared almost immediately. It behooves us, then, in operating, to employ as large and stable a graft as can be obtained.

The number of spines fixed at the time of operation: This has varied from four to eight spinous processes. It has always been our aim to take in the spinous processes of the affected vertebrae and, when possible, at least the spinous processes of two normal vertebrae above and below the affected ones. In marked kyphoses, composed as they are of many diseased vertebrae, this is not always possible, and when accomplished, it is often done at the expense of breaking the graft. But when it is broken, mobility occurs. The fixation is tested in various ways: (1) by a lateral picture of the spinal column sometime after the graft has been embedded; (2) by a leadline tracing of the patient in an upright position and in a

forward bending posture. The results of our cases have shown that the fixation has been complete in 75% of our cases.

Duration of the disease before operation: This plays a very definite rôle as regards the successful outcome of the case. In general, the longer the duration of the disease before the operation, the more likelihood of recovery. In the cases in which the disease had existed less than six months previous to the operation good results were obtained in 37.5% and poor results in 62.5%.

When the disease had existed one year:

Good results — 55%
 Poor results — 45%

When the disease had existed two years:

Good results — 60%
 Poor results — 40%

When the disease had existed over two years:

Good results — 78%
 Poor results — 22%

The real cure of any case of tuberculosis of the spine depends on the amalgamation or fusion of the affected vertebrae and of the affected vertebrae to the normal vertebrae above and below. This takes time. There is no known method which will afford a short cut by which this may be accomplished. Calvé states that in tuberculosis actual ossification that assures repair does not begin until about three years after the onset of the disease. Hence, the cases which have been in existence two years are farther on the road to repair than those of more recent date. The bone graft does not bring about directly consolidation of the vertebral bodies, but only of the spinous processes. But, I believe that it helps indirectly by the formation of a new blood supply and that by its stabilizing effect it alleviates the acute process of the disease.

Duration of post-operative fixation: The after-treatment of Pott's cases recommended by Albee was as follows: The patient is kept in the recumbent position without any plaster jacket or other support for six, eight or ten weeks; he is then allowed to move around without any support, on the assumption that the graft becomes stronger and that too more quickly if the normal physiological activity of the body be permitted. Our experience does not support this view; on the contrary, we believe that proper support must be given in all cases after the operation. In adult cases the period of fixation after operation is much less than in those of infancy and during the years of growth. In the adult case our average was six months, whereas, in infancy and during the period of growth, it was from two and one-half to three years. If this support were not applied, acute exacerbations of the disease soon appeared. It was also found in this growing period that, if a proper support were not worn after the operation, the kyphosis invariably increased, the graft itself was not capable of preventing further deformity; being viable, it was subject to the same

influences and the same laws of strain that had brought about the original kyphos, and instead of preventing a further deformity as growth went on, it bent, becoming more rounded and indeed even acute in outline. In order to prevent the occurrence of a deformity a proper brace or support had to be worn over a long period of time, at least through most of the period of growth.

Influences on abscesses: In general the implantation of a graft has had a beneficial action on the active symptoms of the disease and on the abscess formation. In several cases in which an abscess was present before operation, the rapidity of its disappearance was hastened by the implantation and consequent fixation. On the other hand, abscesses often reappeared when the graft became broken or fixation was not good.

Mortality: Although we have been fortunate enough to encounter no immediate fatality following the operation, the ultimate mortality, properly attributable to it, was eight per cent. The gravity of the operation should make one very careful in the selection of his cases. Children, in infancy and in the growing period, affected with tuberculosis of the spine, and in many cases having other foci of tuberculosis, are not good risks. An extensive procedure of this nature is such a severe drain on their vitality that they are made more liable to a spread of the tuberculosis and to secondary infections, such as influenza, which ultimately prove fatal. The adult, however, is a far better risk and far better able to undergo it.

CONCLUSIONS

In drawing conclusions, therefore, from this series of cases, one can hardly agree with the statement of Dr.

Albee that "Pott's disease must now be regarded as a distinctly surgical affection, the operative treatment a *sine qua non*, to be given precedence over all other therapeutic measures. Mechanical treatment must now occupy a minor position, to be employed only as a post-operative adjunct, or with patients who refuse surgical intervention or are not good surgical risks." My own feeling is that in Pott's disease *operative procedures* should be used only in selected cases, and simply as one of the aids in making a cure, but not as the chief means. Pott's disease is a pathological condition which must necessarily run a very chronic course and its cure is accomplished only when amalgamation of the vertebral bodies is assured. This necessarily takes time. Mechanical means, therefore, particularly in infancy and in the growing child, must be the main method for stabilization of the vertebrae, and when the graft is used it is to be regarded only as an aid and must not be relied upon alone. In infancy the operation is questionable owing to its magnitude and to the tendency which it has to weaken the system and thus favor the incidence of general tuberculosis and other infectious diseases. In this class of cases the ultimate, if not the immediate, mortality is high. In the growing period, from six to sixteen years of age, in carefully selected cases the bone graft may be used, but must be considered only as an aid, and proper mechanical treatment must be kept up until the period of growth is over or amalgamation of the vertebrae has taken place. In adults the bone graft is indicated in the majority of cases, but even here mechanical support should be used for at least six months.

A SUGGESTED MODIFICATION OF THE WRIGHT OPSONIC TECHNIQUE BASED UPON THE DIFFERENTIAL WHITE BLOOD COUNT

By HOWARD B. CROSS

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During the course of an investigation of phagocytosis which was conducted in this laboratory, on several occasions the opsonic index, determined in the manner described by Wright,¹ seemed to be not only an inadequate gauge of the animal's phagocytic defense, but also an indication of a condition quite the opposite of that which was subsequently demonstrated to be the true one. A more detailed analysis of these circumstances was accordingly undertaken in an attempt to define the conditions influencing phagocytosis, but which are not controlled, and often cannot be controlled, in the Wright method of determining the opsonic index. An effort was also made to obtain a factor based upon the differential white blood count which would make the Wright tech-

nique a more dependable method for determining, so far as it is possible to determine, the true phagocytic strength of an animal.

Wright² has demonstrated, by a series of experiments which have been repeatedly confirmed, that the stimulating effect of serum on phagocytosis is directed almost entirely against the bacteria. And it is reasonable to suppose from Bordet's³ work that whenever one bacterium in a medium has been sufficiently opsonized to permit its being taken up by a leucocyte, all similar bacteria of comparable virulence in that environment will likewise be sensitized sufficiently to effect their engulfment. Neglecting, then, the possibility of non-specific absorption of opsonins by leucocytes, the extent of the subsequent

phagocytosis would be determined entirely by the number of phagocytic cells present. Accordingly, it would be impossible to estimate the comparative phagocytic capacity of two animals without taking into consideration not only the white blood count of the experimental animal, but also a differential determination in order to obtain the number of neutrophile leucocytes, the cells most commonly concerned in the phagocytosis of bacteria.

Even by this method of determining a more exact measure of phagocytic capacity, no account is, or can be, taken of the tremendous phagocytic powers of the "fixed" cells. Metchnikoff⁴ was the first to point out that these cells play an important rôle in phagocytosis, and in a more recent investigation Bartlett and Ozaki⁵ have demonstrated that the phagocytic capacity of the "fixed" cells often reveals a compensatory increase whenever there is an exhaustion of the circulating phagocytes. It would seem necessary, therefore, to balance any demonstrable decrease in phagocytic activity of the blood against the possibility that this loss in effectiveness might be compensated for by increased activity of the infinitely more numerous "fixed" cells.

There remains one other factor that should be considered before concluding that a lowered opsonic index, secured according to the method of Wright, indicates an absolute depression in phagocytic defense. It is reasonable to suppose that in consequence of the rapid destruction and replacement of neutrophilic leucocytes during infection that these cellular elements are younger in

infected than in normal animals. Hektoen⁶ suggests that it is possible to account for the increased phagocytic activity, which Tunnick⁷ demonstrated in exudates and in patients recovering from pneumonia, by the fact that the cells obtained under these circumstances are younger than the leucocytes in the blood-stream of normal individuals. This suggests, then, that it would be impossible to demonstrate conclusively a depression in phagocytic effectiveness without first eliminating the possibility of an increased "cytophagic" index which might even over-compensate for the decline in opsonins.

While it is manifestly impossible, by any experimental means whatever, to estimate accurately the total phagocytic capacity of an animal, it is quite useless to employ the Wright technique unless a correction is made for the observed variations in the number of neutrophilic leucocytes present in the experimental animals. The factor by which this correction is made in the following table was obtained in each case by dividing the number of neutrophilic leucocytes present in 1 c. mm. of experimental blood by the corresponding count in the control. The Wright opsonic index is then multiplied by the factor thus derived. This new measure of relative phagocytic capacity may for convenience be designated as the *differential opsonic index*.

The data in the following experiments are intended to show the results of opsonic determinations made, at or a few hours before death, by taking into consideration the differential white blood count of the experimental animals.*

EXP. 1.—The animals recorded in the appended table were infected by intravenous inoculations of the infecting organisms. Sublethal doses were continued until the blood revealed a high bacteriotropic content and then gradually increasing amounts were injected until a killing dose was administered. In each case the duration of the infection was more than 15 days and the animal came to its death in a state of advanced emaciation and complete collapse. The infecting agent was obtained in pure culture from the heart's blood of each animal at death.

Animal	Infecting Organism	NORMAL		EXPERIMENTAL		BACTERIA	FACTOR	Wright † opson. index	Diff.** opson. index
		Average White Count	% Neutroph. leuco.	White Count	% Neutroph. leuco.				
Dog 1	B. bronchisepticus	*10,600	75	27,000	*85	S. aureus	2.88	1.3	3.74
						B. bronchisept.	2.88	1.3	3.74
						B. coli	2.88	1.1	3.06
Dog 2	B. typhosus	10,600	75	3,500	35	S. aureus	0.15	1.1	0.16
						B. bron.	0.15	1.2	0.18
						B. coli	0.15	1.1	0.16
						B. typho.	0.15	9.0	1.35
Dog 3	Pneumococcus Type I	10,600	75	3,000	75	S. aureus	0.28	1.0	0.28
						B. bron.	0.28	1.0	0.28
						B. coli	0.28	1.1	0.30
						Pn. I	0.28	1.0	0.28

* Average counts compiled from results obtained in this laboratory, supplemented by the counts of Klieneberger and Carl.⁸

** Differential opsonic index.

† The Wright opsonic indices recorded in these tables were secured in the manner described by Wright.¹ These data were obtained in an investigation of "The Relation of Phagocytosis to Terminal Infections" published by the author in the Johns Hopkins Bulletin, 1921, XXXII, 350-359.

Exp. 2.—The cats recorded in the following table died of a spontaneous hemolytic streptococcus infection. The onset of the disease was characterized by a violent sneezing and purulent nasal discharge. The animals lost rapidly in weight and died after four to six days in a state of the most profound weakness. Hemolytic streptococci, in pure culture, were cultivated from the blood throughout the course of the infection.

Animal	Infecting Organism	NORMAL		EXPERIMENTAL		BACTERIA	FACTOR	Wright opson. index	Diff. opson. index
		Average White Count	% Neutroph. leuco.	White Count	% Neutroph. leuco.				
Cat 4	Strept. hæmolyticus	14,800	69	50,000	73	S. aureus	3.5	0.9	3.1
						B. bron.	3.5	0.9	3.1
						Str. hæmo.	3.5	0.5	1.7
Cat 5	Strept. hæmolyticus	14,800	69	100,000	90	S. aureus	8.8	0.9	8.0
						B. bron.	8.8	0.9	8.0
						B. coli	8.8	1.3	11.4
						S. hæmo.	8.8	0.8	7.0
Cat 6	Strept. hæmolyticus	14,800	69	12,000	88	S. aureus	1.0	1.0	1.0
						B. bron.	1.0	1.0	1.0
						B. coli	1.0	1.2	1.2
						S. hæmo.	1.0	0.5	0.5

Exp. 3.—The opsonic data recorded in the accompanying table were obtained from a study of human cases, carried out a short time before the death of the patient. The nature and duration of the illness in each case is indicated in the following tabulation:

PATIENT No. 1.—White female; aged 69 years.* Duration of present illness, 8 months. Diagnosis: Arteriosclerosis; hypertension; chronic nephritis; hemiplegia; acute bronchopneumonia.

PATIENT No. 2.—White male; aged 50 years. Duration of present illness: 2 months. Diagnosis: Carcinoma of bladder; hydro-ureters; pyonephrosis; arteriosclerosis with occlusion of coronary arteries; chronic myocarditis.

PATIENT No. 3.—White male; aged 39 years. Duration of present illness: 9 months. Diagnosis: Carcinoma of bladder; operation for removal of carcinoma January 21, 1921. *Streptococcus hæmolyticus* septicemia developed January 26. Patient died the following day.

PATIENT No. 4.—Colored female; aged 32 years. Duration of present illness: 9 months. Diagnosis: Carcinoma of cervix with metastasis to brain; pyelitis; bronchopneumonia; syphilis.

* The clinical data of the following human cases were secured from The Johns Hopkins Hospital records.

DATE	REMARKS	NORMAL		EXPERIMENTAL		BACTERIA	FACTOR	Wright opson. index	Diff. opson. index
		Average White Count	% Neutroph. leuco.	White Count	% Neutroph. leuco.				
3/2/21	Pat. 1	5,500	69	11,400	74	S. aureus	2.2	1.0	2.2
						B. coli	2.2	1.0	2.2
						B. bron.	2.2	1.0	2.2
						Pn. I	2.2	1.0	2.2
1/3	Pat. 2	5,500	69	19,300	73	S. aureus	3.7	1.5	5.5
						B. coli	3.7	1.3	4.8
						B. bron.	3.7	1.2	4.4
						B. typho.	3.7	0.9	3.3
1/28	Pat. 3	5,500	69	32,840	89	S. aureus	7.7	0.9	6.9
						B. coli	7.7	0.9	6.9
						B. bron.	7.7	1.3	10.0
						S. hæmo.	7.7	0.1	0.8
2/11	Pat. 4	5,500	69	24,000	75	S. aureus	4.7	0.9	4.2
						B. coli	4.7	0.8	3.7
						B. bron.	4.7	1.2	5.6
						B. typho.	4.7	1.0	4.7

A study of the tables in Experiments 1 and 2 reveals that an actual depression in phagocytic activity associated with infectious diseases is not an invariable phenomenon and, indeed, is probably rare except in individuals with a low leucocytic count. In most cases the increase in neutrophilic leucocytes over-compensates for the apparent decrease in opsonic efficiency revealed by the Wright technique. It seems reasonable, then, to suppose that infections may triumph not because of a rupture in the phagocytic activity of the animal but in spite of a very considerable increase in the effectiveness of that defensive mechanism. Likewise, an examination of the table in Experiment 3 shows that an increase in neutrophilic leucocytes often insures a high differential opsonic index in chronic diseases of an infectious as well as a non-infectious nature.

The opsonic index and differential index are, of course, the same in all cases where the experimental animal has a normal leucocytic count. The two indices may even be the same when either a high or low white count is obtained, provided the opsonic index varies equally in the opposite direction. In general, however, the variation in the white count presents a greater range than is observed in the opsonic indices, so that the value of the differential index will, as a rule, be influenced more by the white count than by the observed opsonic index. It does not, therefore, seem unreasonable to suppose, that in any attempt at estimating an animal's phagocytic capacity, it is quite as important to obtain a differential blood count as it is to determine the opsonic index. These factors are interdependent, and both alike are indispensable in any calculation designed to evaluate an animal's phagocytic defense.

It is, of course, recognized that any attempt at interpreting phagocytic tests conducted outside the animal body must be undertaken with considerable reservation. There is no method for controlling possible variations in the phagocytic capacity of the "fixed" cells, and there is always the possibility that in "test-tube" phagocytosis one may be dealing with the enfeebled and non-pathogenic

organisms that occur in even the most virulent cultures. In this connection Bordet⁸ has pointed out that, when highly virulent bacteria are injected intraperitoneally, there is always an initial phagocytosis of what he regards as the weaker and less aggressive organisms. The more virulent fraction of the cultures resists engulfment and carries the infection to a fatal conclusion. If, then, in *in vitro* phagocytic tests the bacteria taken up represent largely the indifferent, non-aggressive fraction of the culture, such results would be of little value in determining the true extent of an animal's phagocytic defense.

CONCLUSIONS

1. The Wright opsonic index is not a reliable measure of an animal's phagocytic capacity whenever the white blood count is above or below the normal.
2. The *differential opsonic* index seems generally to be a more dependable measure of phagocytic defense than the Wright opsonic index.
3. Depression in phagocytic activity is not an invariable phenomenon associated with fatal infections.
4. With the possible exception of cases with leucopenia, the relative phagocytic power of an infected animal is never lower than the opsonic index indicates, but is sometimes, indeed quite often, very much higher. The opsonic index, with the exception noted above, never does more than express the minimal limitations of the animal's phagocytic defense.

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THE DISSEMINATION OF BACTERIA IN THE UPPER AIR PASSAGES II.—THE CIRCULATION OF BACTERIA IN THE MOUTH*

By ARTHUR L. BLOOMFIELD

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In the preceding paper of this series¹ experiments were reported on the mechanism of removal of charcoal particles placed on various areas of the mouth and throat. It was found that such particles were removed in a definite and orderly way, along direct paths leading back to the posterior tongue and oesophagus.

*Read before The Johns Hopkins Hospital Medical Society, November 7, 1921.

The present communication deals with similar experiments in which bacteria were used as a test substance.

METHODS

Sarcina lutea was used as the test organism. Its advantages for our purpose were as follows: (1) It is absolutely non-pathogenic for human beings; (2) The bright yellow colonies can be easily identified in cultures; (3)

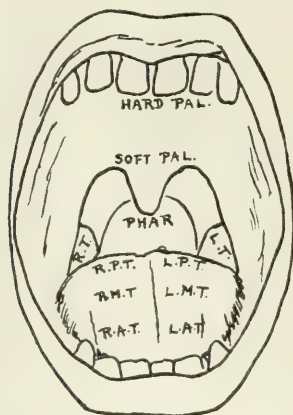
The organism grows well in symbiosis with the usual mouth bacteria.

Healthy volunteers were used as subjects. Solid masses of twenty-four-hour growths of sarcina on agar were inoculated on various areas in the mouth and throat and cultures made as indicated below.

The abbreviations used are as follows:

L. } A. T. = Left	} anterior part of tongue
R. } = Right	
L. } M. T. = Left	} middle part of tongue
R. } = Right	
L. } P. T. = Left	} posterior part of tongue
R. } = Right	
Rt. } Ton = Right	} tonsil
Lt. } = Left	
Phar = Posterior pharyngeal wall	
Hard Pal. = Hard palate	
Soft Pal. = Soft palate	

The location of these areas is indicated in the following diagram:



EXPERIMENT 1. Subject H. This person was a laboratory worker in good health. The mouth and throat appeared normal. The tonsils were small and moderately well protected by the anterior pillars.

Nov. 2, 1921. One large platinum loop of a twenty-four-hour growth of sarcina lutea on agar was inoculated on the anterior central part of the tongue. Cultures were made after ten minutes. The number of colonies recovered from various areas was as follows:

A. T.	=	∞	
M. T.	=	∞	
P. T.	=	∞	
Hard Pal.	=	10	
Soft Pal.	=	2	
Pharynx	=	0	
Rt. Ton.	=	10	Lt. Ton. = 14

COMMENT: The bacteria adhered at the site of inoculation and were not generally disseminated through the mouth cavity. There was slight transfer to the hard palate by direct contact. The organisms were drawn back along the tongue and a very few reached the tonsils and soft palate by contact. None were recovered from the pharynx. The point of special

importance is that innumerable colonies progressed along the posterior tongue, whereas only a very few were implanted on the tonsils.

EXPT. 2. Subject B. This person was a healthy laboratory worker. There was no special abnormality of mouth or throat. The tonsils were small, but not well protected by the anterior pillars.

Oct. 26, 1921. One large loop of a twenty-four-hour culture of sarcina lutea on agar was inoculated on the left anterior portion of the tongue. Cultures were made after ten minutes. The number of colonies recovered from various areas was as follows:

L. A. T.	=	∞	R. A. T.	=	0
L. M. T.	=	∞	R. M. T.	=	0
L. P. T.	=	400	R. P. T.	=	25
Lt. Ton.	=	25	Rt. Ton.	=	0
			Soft Pal.	=	0
			Pharynx	=	600

COMMENT: The bacteria adhered at the site of inoculation and were not generally disseminated through the mouth cavity. The organisms travelled back along the homolateral side and did not cross the midline until the posterior tongue was reached when a few were recovered from the opposite side. In spite of the fact that six hundred colonies were recovered from the pharynx (probably directly implanted from the posterior tongue) only a few organisms were found on the tonsil.

EXPT. 3. Subject H. This patient was a healthy physician. The tonsils had been cleanly removed. The tonsil fossæ were shallow. The throat appeared normal.

Oct. 27, 1921. One large loop of a twenty-four-hour culture of sarcina lutea on agar was inoculated on the left tonsillar fossa. Cultures were made after ten minutes. The number of colonies recovered from various areas was as follows:

		Hard Pal.	=	7	
L. A. T.	=	0	R. A. T.	=	0
L. P. T.	=	∞	R. P. T.	=	51
Lt. Soft Pal.	=	120	Rt. Soft. Pal.	=	34
Lt. Ton.	=	∞	Rt. Ton.	=	0
		Pharynx	=	∞	

COMMENT: There was considerable spread, evidently by direct contact, from the tonsil fossa to pharynx and tongue on the same side. Only a few organisms reached the palate and the posterior part of the tongue on the opposite side.

EXPT. 4. Buck, Ward M. This patient was convalescent from typhoid fever. He had small clean deep-set tonsils well protected by the anterior pillars. The throat appeared normal.

Nov. 2, 1921. One large loop of a twenty-four-hour growth of sarcina lutea on agar was placed on the right tonsil well up and behind the anterior pillar. Cultures were made after ten minutes. The number of colonies recovered from various areas was as follows:

L. A. T.	=	0	Hard Pal.	=	0	R. A. T.	=	0
L. M. T.	=	0				R. M. T.	=	0
L. P. T.	=	0				R. P. T.	=	0
Lt. Ton.	=	0				Rt. Ton.	=	∞
Lt. Pharynx	=	0	Soft Pal.	=	0	Rt. Pharynx	=	0

COMMENT: No sarcina lutea was recovered from any area save the site of inoculation. This experiment should be contrasted with Experiment 3 to emphasize the importance of anatomical variations in allowing spread by direct contact.

EXPT. 5. Subject C. A healthy medical student. No special abnormality of throat. Moderate-sized clean tonsils.

Nov. 2, 1921. A large loop of a twenty-four-hour growth of *sarcina lutea* on agar was inoculated on the posterior pharyngeal wall. Cultures were made after ten minutes. The number of colonies recovered from various areas was as follows:

L. A. T. = 0	Hard Pal. = 0	R. A. T. = 0
L. M. T. = 0		R. M. T. = 0
L. P. T. = 0		R. P. T. = 0
Lt. Ton. = 30	Soft Pal. = 1	Rt. Ton. = 35
	Pharynx = 340	

COMMENT: It may be noted that the organisms did not come forward into the mouth at all. The few colonies from the tonsils and soft palate were clearly transferred by direct contact. There was a rapid disappearance from the pharynx.

SUMMARY OF EXPERIMENTS 1—5

The following points may be noted.

1. *Sarcina lutea* inoculated on various areas in the mouth and pharynx adhered at the site of inoculation and was not uniformly spread over the mouth cavity.

2. There was some transfer to areas other than the site of inoculation by direct contact varying with anatomical conditions (e. g., tongue to hard palate, tonsil to posterior tongue and soft palate, etc.).

3. Aside from such limited spread by direct contact, the removal of the organisms was affected by a progression towards the esophagus.

4. Organisms inoculated on one side of the tongue travelled along the same side with practically no spread across the midline.

5. Organisms inoculated on the tonsil or pharynx did not come forward into the mouth.

6. These experiments agree entirely with those previously described in which charcoal particles were used instead of bacteria.

In the next series of experiments the object was to determine to what extent organisms implanted on the mucous surface were removed or spread about by artificial flushing of the mouth and throat.

EXPT. 6. Subject H. (see EXPT. 3).

Oct. 29, 1921. One loop of a twenty-four-hour growth of *sarcina lutea* on agar was placed on the left anterior portion of the tongue. Immediately after the inoculation the subject drank a large glass of water. Cultures were thereupon made. The number of colonies recovered from various areas was as follows:

L. A. T. = ∞∞	Hard Pal. = 35	R. A. T. = 0
L. M. T. = 34		R. M. T. = 0
L. P. T. = 45		R. P. T. = 0
Rt. Ton. = 0	Soft Pal. = 3	Lt. Ton. = 0
	Pharynx = 2	

COMMENT: It may be noted that no spread could be determined following the flushing, save for the three colonies on the soft palate and the two from the pharynx. The slight spread to the hard palate was probably by direct contact.

EXPT. 7. Subject B. (see EXPT. 2).

Oct. 31, 1921. A large loop of a twenty-four-hour growth of *sarcina lutea* was inoculated on the posterior central part of the tongue. A large glass of water was rapidly sipped im-

mediately after inoculation. Cultures were thereupon made from various areas. The number of colonies recovered was as follows:

L. A. T. = 0	Hard Pal. = 4	R. A. T. = 0
L. M. T. = 0	Soft Pal. = 15	R. M. T. = 0
L. P. T. = ∞∞		R. P. T. = ∞∞
Lt. Ton. = 300		Rt. Ton. = 20
	Pharynx = 5	

COMMENT: It may be noted that no *sarcina lutea* was recovered in front of the inoculated area save four colonies from the hard palate. The slight spread to soft palate and tonsils was probably by direct contact. Innumerable colonies of *sarcina* were however recovered from the site of inoculation.

EXPT. 8. Subject H. (see EXPT. 1).

Oct. 31, 1921. One large loop of a twenty-four-hour growth of *sarcina lutea* was inoculated on the right tonsil. A large glass of water was immediately taken. Cultures were thereupon made from various areas. The number of colonies of *sarcina lutea* recovered was as follows:

L. A. T. = 0	R. A. T. = 0
L. M. T. = 0	R. M. T. = 0
L. P. T. = 0	R. P. T. = 3
Lt. Ton. = 0	Rt. Ton. = 800
Lt. Pharynx = 1	Rt. Pharynx = 0
Lt. Soft Pal. = 0	Rt. Soft Pal. = 0

COMMENT: Practically no *sarcina lutea* was recovered save from the site of inoculation.

EXPT. 9. Subject Hu. This subject was a healthy physician. He had a very narrow palate arch and the posterior pillars were practically in contact with the pharyngeal wall.

Oct. 29, 1921. One loop of a twenty-four-hour growth of *sarcina lutea* was inoculated on the right posterior pharyngeal wall. A glass of water was immediately taken and cultures were thereupon made from various areas. The number of colonies of *sarcina lutea* recovered was as follows:

L. A. T. = 0	Hard Pal. = 0	R. A. T. = 0
L. M. T. = 0		R. M. T. = 0
L. P. T. = 0		R. P. T. = 2
Lt. Ton. = 0		Rt. Ton. = 0
Lt. Pharynx = 800	Soft Pal. = 12	Rt. Pharynx = 2000

COMMENT: A few colonies of *sarcina* were recovered from the soft palate and right posterior tongue—evidently transferred from the pharynx by direct contact.

SUMMARY OF EXPTS. 6—9

The following points may be noted:

1. *Sarcina lutea* thoroughly implanted on various areas in the mouth and pharynx adhered at the site of inoculation.

2. The ingestion of a large glass of water following inoculation did not seem to remove more than a very few of the organisms.

3. Whatever the number removed, such organisms were swallowed with the ingested fluid and not reimplanted on other parts of the mouth or throat save for an occasional colony.

4. These experiments correspond with the findings in the experiments with charcoal particles.

In the next experiments an attempt was made to determine to what extent organisms ingested in a fluid medium contaminated the mouth cavity.

EXPT. 10. Subject B. (see EXPT. 2).

Nov. 2, 1921. A suspension of sarcina lutea in saline solution was prepared, one loop of a twenty-four-hour growth on agar to each cubic centimeter of fluid. The subject drank about 30 c.c. of this suspension. Cultures were immediately made from various areas. The number of colonies of sarcina recovered was as follows:

Anterior Tongue	=	50
Left sublingual space	=	6
Posterior tongue	=	∞
Soft Palate	=	8
Hard Palate	=	9

Rt. Ton. = 34 Phar. = 9 Lt. Ton. = 33

COMMENT: It may be noted that only a minute portion of the ingested organisms adhered. Except from the posterior part of the tongue only negligible numbers were recovered.

EXPT. 11. Subject, Buck. (see EXPT. 4).

Nov. 2, 1921. The subject drank about 30 c.c. of a suspension of sarcina four times as heavy as that used in Expt. 10. Cultures were immediately made. The number of colonies of sarcina recovered was as follows:

Sublingual space	=	175
Hard Palate	=	66
Anterior Tongue	=	4000
Posterior Tongue	=	1600
Soft Palate	=	8

Rt. Ton = 60 Phar. = 14 Lt. Ton. = 12

COMMENT: The result of this experiment is similar to that obtained in Expt. 10.

SUMMARY OF EXPTS. 10 and 11

The following points may be noted:

1. After the subject had drunk a heavy suspension of sarcina, relatively few organisms adhered to the mucous membranes.

2. The greatest number was recovered from the tongue, a few adhered to the tonsils, and practically none to the posterior pharyngeal wall.

DISCUSSION

The experiments recorded above agree completely with the results obtained in the previous work with charcoal particles. It appears, in the first place, that the mouth is not a churn whereby entering organisms are more or less uniformly spread over its surface. On the contrary, even when introduced in a fluid medium, they are carried directly back by the act of swallowing with but little contamination of, or at least little tendency to adhere to, the tonsils, pharynx, or sublingual space. Once anchored, however, in the mucous lining of the buccal cavity the organism is no longer readily dislodged, at least by flushing with fluid. It is drawn rapidly back by suction currents which tend to avoid the tonsils and posterior pharynx. These currents act in a constant and orderly manner and are disturbed only by anatomical variations which may lead to some spread of organisms by direct contact.

It seemed desirable to extend the above experiments by using pathogenic organisms, but we did not wish to subject the volunteers to the danger of possible infection. Our purpose was accomplished indirectly in another way.

It appeared that carriers who harbored pathogens in a localized area might yield the desired information. The following observations were therefore made:

CASE I. This subject was a clinically healthy individual who had been under observation for over a year. Numerous cultures had been made which indicated that he was a tonsil carrier of *Staphylococcus aureus*. Conditions were therefore analogous to an experiment in which this organism was inoculated on the tonsil save that in this case the inoculation came from foci of infection in the tonsil itself. Cultures were made from various areas in the mouth and throat and the number of colonies of *S. aureus* recovered were noted. The results were as follows:

Site of Culture Date	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
Mar. 21, 1921	100	200	0	0
Mar. 23, 1921	∞	∞	2	0
Mar. 28, 1921	∞	∞	0	0
Apr. 16, 1921	50	∞	4	0
May 15, 1921	∞	4	1	0

It may be noted that, although the staphylococci were constantly present in large numbers on the tonsils, none were recovered from the tongue, and only an occasional colony from the pharynx. The results were similar to those obtained in the experiment in which sarcina was placed on the tonsil. More detailed cultures from the same person yielded the following results:

Oct. 28, 1921. Number of colonies of *S. aureus* recovered from various areas:

L. A. T.	= 0	Hard Pal.	= 0	R. A. T.	= 0
L. M. T.	= 3	Soft Pal.	= 23	R. M. T.	= 0
L. P. T.	= 6	Pharynx	= 1	R. P. T.	= 10
Lt. Ton.	= 15			Rt. Ton.	= 50

Oct. 31, 1921:

L. A. T.	= 0	Hard Pal.	= 0	R. A. T.	= 0
L. M. T.	= 0	Soft Pal.	= 3	R. M. T.	= 0
L. P. T.	= 0	Pharynx	= 0	R. P. T.	= 1
Lt. Ton.	= 50			Rt. Ton.	= 3

CASE II. Bu. This patient was a tonsil carrier of *Staphylococcus aureus*. Cultures made from various areas yielded colonies of this organism as follows:

Nov. 3, 1921:

L. A. T.	= 0	Hard Pal.	= 0	R. A. T.	= 0
L. M. T.	= 0	Soft Pal.	= 0	R. M. T.	= 0
L. P. T.	= 1			R. P. T.	= 0
Lt. Ton.	= 200			Rt. Ton.	= 7
Lt. Pharynx	= 0			Rt. Pharynx	= 0

CASE III. Go. This patient had acute nephritis. He was found to be a carrier of *B. influenzae*. Differential cultures showed this organism as follows:

Site of Culture Date	Rt. Tonsil	Lt. Tonsil	Pharynx	Soft Palate	Tongue
Nov. 5, 1921	0	0	∞	0	0
Nov. 7, 1921	0	0	200	0	0

It may be noted that influenza bacilli, while present in large numbers in the pharynx, were not spread to other areas in the buccal cavity.

CASE IV. H. This patient had peripheral neuritis. He, also, was a carrier of *B. influenzae*. Differential cultures showed this organism as follows:

Site of Culture Date	Rt. Tonsil	Lt. Tonsil	Pharynx	Soft Palate	Tongue
Nov. 7, 1921	0	0	∞	0	0
Nov. 5, 1921	0	0	∞	0	0

The result is similar to that in Case III.

CASE V. No. This person was a healthy medical student. He was found to be a carrier of *B. influenza*. Differential cultures showed this organism as follows:

Site of Culture Date	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
Aug. 20, 1921	Several hundred	Several hundred	0	0
Aug. 26, 1921	150	6	0	0
Sept. 7, 1921	Several hundred	∞	0	0

This case is in interesting contrast to Cases III and IV. In the former influenza bacilli were localized in the pharynx and were not spread elsewhere. In Case V they were localized in the tonsils and were not spread to the pharynx.

CASE VI. F. This subject was a physician convalescent from acute tonsillitis. He was found to be a carrier of a hemolytic streptococcus. Differential cultures yielded this organism as follows:

Site of Culture Date	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
Mar. 12, 1921	200	100	∞	0
Mar. 18, 1921	∞	∞	∞	0
Mar. 16, 1921	∞	∞	∞	0
Mar. 14, 1921	∞	Many	∞	0
Mar. 29, 1921	Several hundred	Many	4	0
Apr. 12, 1921	Many	Many	∞	0
May 13, 1921	Several hundred	Several hundred	50	0

It may be noted that while this person constantly had large

numbers of streptococci on the tonsils and pharynx, none were ever recovered from the tongue.

The cases presented above are merely a few of many observations which may serve as examples. They show clearly that in spontaneous inoculations from a local focus of infection the same laws of spread hold as in the experimental inoculations.

GENERAL DISCUSSION

The experiments described in this paper and in the preceding one show clearly that there is a definite mechanism whereby foreign organisms which enter the mouth are removed. Its essential feature is a direct and rapid transport of the bacteria towards the œsophagus. Without having further experimental proof it appears probable that the organisms are swallowed after reaching this point.

It does not seem wise at present to go too far in ascribing a purposeful significance to this mechanism of elimination. The causes which lead to its failure as well as the question of other factors, beside the purely mechanical one, which may enter into the protective mechanism of the upper air passage, will be discussed at another time.

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BACTERIAL NUTRITION

GROWTH OF A HEMOPHILIC BACILLUS ON MEDIA CONTAINING ONLY AN AUTOCLAVE-STABLE SUBSTANCE AS AN ACCESSORY FACTOR

By T. M. RIVERS, M. D.

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It has been shown by a number of investigators¹⁻⁵ that two factors are essential for the growth of influenza bacilli. One of these is autoclave-stable, the other is autoclave-labile. Both are found in blood and also in substances which contain no blood.^{6,7} Speculation as to the exact nature of these two substances and how they act to promote growth is rife. Definite proof, however, for none of these contentions has been produced.

In this laboratory four groups of so-called hemophilic bacilli have been studied to determine their requirements in regard to these two growth accessory factors—the non-hemolytic influenza bacillus, the hemolytic influenza bacillus, the pertussis bacillus and a hemophilic bacillus isolated from the preputial secretions of dogs. The non-hemolytic group answers all the old criteria for influenza bacilli. The hemolytic ones correspond to the bacilli described by Pritchett and Stillman,⁸ Rivers,⁹ Stillman and Bourn,¹⁰ and Rivers and Leuschner.¹¹ The pertussis bacillus (No. 256) was supplied by the Hygienic Laboratory in Washington. It does not produce indole, does

not reduce nitrates, does not ferment any of the ordinary sugars and makes milk strongly alkaline. The bacilli isolated from the preputial secretions of dogs have been collected from animals in the laboratory during the last two years and correspond to *B. hemoglobinophilus canis* first described by Friedberger.¹² This group is very interesting and will be taken up in detail in another paper. Only the growth requirements will be discussed here.

The growth accessory factors were prepared in the following manner:

A. Autoclave-labile Factor.—A pure culture of brewer's yeast was grown on malt agar, pH 5-6, scraped off and washed in physiological salt solution three times. To the yeast cells from 5 to 10 volumes of distilled water were added, brought to a boil, allowed to stand on ice over night, filtered through paper and sterilized by means of a Mandler filter.

B. Autoclave-stable Factor. Hematin.—Red blood cells from 100 c.c. of blood were washed three times in physio-

logical salt solution, laked with distilled water and precipitated with 95 per cent alcohol. The precipitate was boiled with weak acid alcohol (H_2SO_4) until colorless and then filtered. The filtrate was partially saturated with NaCl and filtered. The precipitate was washed thoroughly on the filter with distilled water, dissolved in warm acid alcohol, reprecipitated with NaCl, filtered, washed, taken up in acid alcohol, made slightly alkaline (pH 7.4) with NaOH and brought up to volume of 100 c.c. with 95 per cent alcohol. While hematin prepared in this way may not be absolutely pure, it is relatively so and answers the purpose desired in this work.

Two basic media, to which the growth accessory factors could be added separately or together, were used. One was meat-infusion agar, the other was made up with 2 per cent agar, 2 per cent Fairchild's peptone and 0.5 per cent NaCl. The autoclave-stable factor was added to the basic media in quantities of 2 c.c. per 100 c.c. and always autoclaved. The labile factor, 15 c.c. per 100 c.c., was added to the melted agar after it had cooled down to 50°C.

TABLE I.—GROWTH ACCESSORY REQUIREMENTS OF CERTAIN SO-CALLED HEMOPHILIC BACILLI.

Bacterium	Meat Infusion Agar	Meat Infusion Agar + Hematin	2 per cent Peptone Agar	2 per cent Peptone + Yeast Ext.	2 per cent Peptone Agar + Hematin	2 per cent Peptone Agar + Yeast Ext. + Hematin
<i>B. pertussis</i>						
No. 256	+	+	+	+	+	+
<i>B. hemoglobinophilus canis</i> (isolated 1 yr.)	—	+	—	—	+	+
<i>B. hemoglobinophilus canis</i> (isolated 1 wk.)	—	+	—	—	+	+
<i>B. influenzae</i> , non-hemolytic (isolated 7 yrs.)	—	—	—	—	—	+
<i>B. influenzae</i> , hemolytic (isolated 2 yrs.)	—	—	—	—	—	+

+ indicates that successful transplants were made as long as desired. — indicates that successful transplants were not obtained or for one or two generations only.

From Table I it can be seen that *B. pertussis*, No. 256, requires neither of the growth accessory factors; the hemolytic and the non-hemolytic influenza bacilli require both, the recent and the old isolations of the bacillus from dogs require the addition of only the autoclave-stable factor.

DISCUSSION

Fildes³ made a peptic digest of blood. The hematin settled to the bottom leaving a clear straw-colored supernatant fluid. After the precipitate and supernatant fluid were properly separated, he found that *B. influenzae* did not grow on a medium to which these substances were added separately. A good growth, however, occurred

when they were used together. From these and other observations he concluded that the combined oxygen, probably in the form of a peroxide, was activated for the influenza bacillus by the catalytic action of iron in the blood pigment. He did not state in which part of the medium the loosely bound oxygen was found, but it may be inferred that it was in the clear supernatant fluid of the peptic digest of blood. If this inference be correct, an interaction of the two parts of the digest was necessary for the growth of influenza bacilli. Davis² in speaking of the heat-stable and heat-labile factors says: "The interaction of these two substances is somehow necessary for the growth of this organism. Presumably the second factor in some way renders the iron more available and, in view of the nature and function of this element in life processes, one is tempted to interpret the phenomenon as related to oxidation, and possibly catalytic in nature." The above investigators seem to think some interaction between the two accessory factors takes place, but it is evident that they do not agree upon the type of interaction. Thjötta and Avery⁷ in the report of their work on the two factors say, "They are both requisite for growth of hemophilic bacilli and each is separately inactive and without effect." From this it might be inferred that they consider some interaction between or coaction by these two substances necessary. Olsen,¹³ Fildes,³ and Thjötta and Avery,⁷ think that the positive guaiac or benzidine tests given by the suitable factors for growth are very significant, yet the latter workers admit that bananas, although giving a negative benzidine reaction, support growth of *B. influenzae*.

It has been shown that *B. hemoglobinophilus canis* requires the addition of only an autoclave-stable substance as an accessory growth factor. This observation certainly makes one desire more proof that there is actually an interaction or coaction between the two factors involved in the growth of influenza bacilli. Both are undoubtedly essential, but it does not necessarily follow that there is any interaction. It is conceivable that they may have nothing to do with each other in the growth of hemophilic bacilli. It is also possible that the autoclave-stable factor acts in the same manner in both instances; in the case of influenza bacilli something else, which may or may not have anything to do with the action of the autoclave-stable factor, is necessary; in the case of *B. hemoglobinophilus canis* nothing else is essential or, if there be, it is synthesized by the organism. It is fully realized that what transpires in the growth of one group of hemophilic bacilli is not entirely analogous to what occurs in others. Still the fact that a certain group requires the addition of only one of the accessory factors throws doubt on any theory of interaction between the two factors to produce growth in other closely allied groups until more definite proof is produced.

CONCLUSIONS

A hemophilic bacillus has been found which requires the addition of only an autoclave-stable substance as a growth accessory factor.

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ON THE PRESENCE OF NUCLEIC ACID IN BACTERIA

(A PRELIMINARY REPORT)

By ALEXANDER J. SCHAFER, CASPAR FOLKOFF AND
S. BAYNE-JONES.

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The authors have not been able to find in the literature any report of the isolation of nucleic acid from bacteria. Ruppel, in 1898, in a chemical study of the tubercle bacillus, reported the preparation of a white powder, which he found to contain the amount of phosphorus theoretically required for nucleic acid (9.42%). But this substance gave a biuret reaction, and no other procedures for the actual identification were resorted to. We thought, therefore, that the actual demonstration of nucleic acid and its isolation were desirable.

Since two different nucleic acids have been distinguished as types of animal and plant nucleic acid, it occurred to us that the identification of the variety present in bacteria, if any were found, would be of additional interest from a taxonomic, if from no other, standpoint.

Bacteria were grown in mass upon a synthetic medium which contained no trace of formed purine or pyrimidine compounds. The following medium, upon which the organism used (*B. coli*) grew well, was devised:

Agar	20.0 gm.
Sodium chloride	5.0 gm.
Calcium chloride	0.1 gm.
Magnesium sulphate	0.2 gm.
Dipotassium phosphate ...	0.2 gm.
Ammonium lactate	12.0 gm.
Glycerine	30.0 cc.
Water	1000 cc.

The medium was titrated to P_h 7.8

B. coli communior was used because of its facile growth and comparative non-pathogenicity. The method of growth and preservation of the bacteria will be described in a later paper.

When approximately 500 grams of dehydrated bacteria had been accumulated, the nucleic acid was isolated. For this we employed with slight modification the unpublished method which Walter Jones and Folkoff applied to the isolation of nucleic acid from yeast, but which does not lead to the isolation of nucleic acid from animal tissues.

The substance finally obtained consisted of a non-hygroscopic, protein-free, slightly gray, amorphous powder. It contains closely the amount of phosphorus required for plant nucleic acid, half of which is easily split and half firmly bound. It has thus far been shown to contain guanine, which it produces by the action of the mildest hydrolytic processes, and is surely to be regarded as a substance belonging to the group called nucleic acid. But the material does not contain the crucial pentose group of plant nucleic acid, since it does not respond in the slightest degree to the sensitive color reactions with phloroglucine and orcinic.

We propose to isolate this substance from bacteria in amount sufficient for its close chemical examination.

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THE RÔLE OF SITUATION IN PSYCHOPATHOLOGICAL CONDITIONS. (ABSTRACT.)

By ESTHER LORING RICHARDS

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Mental Hygiene, Vol. 5, No. 3, pp. 444-467. July, 1921.

The material for this paper was taken from the Out-patient Department of the Henry Phipps Psychiatric Clinic of the Johns Hopkins Hospital. It consists of a study of seventeen children who were referred in September and October, 1920, for various types of "nervousness." Inasmuch as over sixty per cent. of the new admissions to this Dispensary every month are children, it seemed worth while to follow a group of cases intensively over a six months' period and see what practical therapeutic helps might be derived from such work. These children were not approached with the unconscious determination to find in them an exposition of any preconceived theory, or general cause for disorders of balance as a whole. Each patient was studied as an individual child with his own story of etiology and constructive assets. In every instance the physical condition was passed upon by one or more physicians on the general dispensary staff of the hospital. In addition to data obtained from the social organization, the school, or dispensary referring the child, each case was carefully studied by one of our psychiatric social workers. The latter made repeated visits to the home and school, listening to the stories of family and teachers, and effecting that exchange of viewpoints which makes for mutual understanding and intelligent co-operation. The material obtained in this study was tabulated under the headings of Complaint, Physical Status and Mental Level, Situational Data, Suggested Modifications, and Follow-up Notes. In every case the light shed on the "Complaint" did not come from somatic and psychological facts, but from the data of situation. For example:—

C. K. (No. 1) is a boy of eight who was brought to the dispensary by his mother with the complaint that he was "backward in everything." He was teased by younger children, would give up his rights sooner than fight or argue, and sat around the house doing nothing after school. The boy slept poorly and was afraid of everything.

Examination of Patient and Situation: The patient was pronounced physically normal in the Harriet Lane Dispensary. He had a mental age of eight years plus, according to the Binet-Simon tests. In appearance he seemed bright and full of normal interests. He was in the third grade of a small private school, not being allowed to go to the public school because it was "too rough." He owned a football, a baseball, and a bat, all of which were in good condition because he was allowed to use them only in the small backyard of his home.

Apparently he had never had an opportunity to receive a black eye or get his feet wet. On investigation it developed that the father and mother were separated, and the latter and the patient lived with the maternal grandmother. The father was a ne'er-do-well and alcoholic, and the mother felt that the patient should be shielded from influences that might tend to develop inherited characteristics. Hence his companions and play had been carefully supervised. The child felt in some vague way that he was different from other children, and that this difference was associated with the father, who must have been a very bad man. The mother ascribed the patient's odd behavior to "nerves," which (she thought) were a sure sign of tainted inheritance.

Suggested Modification and Results: An attempt was made to show the mother that this child's faulty characteristics were the product of her own solicitude and fear. It was suggested that she try a program of encouraging the patient's initiative—transfer to a larger school, right of way to playground without continued watching and fear of disaster.

The mother reports at the end of five months that the patient has not only outgrown his previous tendencies, but seems to have made up for lost time in his noisiness and irrepressibility. In appearance the boy is franker and more communicative. He speaks voluntarily of the part he is taking in games, and boasts of his prowess in having settled a personal dispute or two with "fellers bigger than I am."

The complaints for which these children were referred—twitching, retardation, speech disturbance, excitability, imaginary somatic distress, etc.—may or may not augur ill for the future. For the present, at least, they constitute a handicap to the start in life that cannot be ignored. The presence of "spells," for example, whether temper storms or pseudo-convulsive tricks, tends to single out the child from other children in the family and other scholars in the classroom. The watching, and anxiety have its effect on the child himself. In some instances it fosters embarrassment with feelings of inferiority; in others it acts as a wedge for native aggressiveness and lack of consideration; and in every instance it focuses the attention of the child on himself with a wealth of unhealthy reactions so familiar in the psychopathology of everyday life. In reviewing the background of situations from which the above-mentioned complaints have arisen, one is impressed by the absence of any great and startling facts, especially if one has been accustomed to

associating psychopathological conditions with definite etiological factors—such as somatic disease, emotional strains of shock and conflict, and desperate adaptive problems of poverty and hardship. The situational data presented here involve such facts as poor habit training, faulty understanding of the individual child, and un-

wholesome attitudes of parents and teachers expressed in chronic worry, over-solicitude, nagging, repression of initiative, too much stimulation, pushing beyond native capacities, etc. Petty and commonplace facts they seem in the telling, and yet capable of producing tremendous influence on the springs of human activity.

NOTES ON NEW BOOKS

Diseases of the Chest and the Principles of Physical Diagnosis.

By GEORGE WILLIAM NORRIS and HENRY R. M. LANDIS. With a Chapter on the Electrocardiograph by Edward B. Krumbhaar. (W. B. Saunders Company, 1920, Philadelphia and London.)

It is a difficult matter to write a practical book on the physical diagnosis of the heart and lungs in health and disease. But this, the expressed purpose of the authors, has certainly been well accomplished, and any criticisms here made should be applied not to the authors' work, but rather should be regarded as in the nature of suggestions that have been derived from the constant use of the book. It is the text-book in use at The Johns Hopkins Medical School for the third year students, and supplies their needs better than any other available book on Physical Diagnosis. But in the hands of second year men there are difficulties not yet overcome. Perhaps in the near future there may be arrangements of medical school curricula that will permit the study of the normal

hearts and lungs in immediate connection with the first year work in anatomy and physiology. When that day arrives, it may be that satisfactory books will be written that will be more useful than those now existing—a combination of physiology, anatomy, physics, and physical diagnosis. So much in physical diagnosis requires a trained physicist that the text-books of today, written as they are by medical men, are rather loose in their applications of physical theories to the human body. This book, however, has one particularly pleasing characteristic, the result of the work of the anatomist, George Fetterhoff. His frozen-section work with the excellent plates supplies a unique demonstration of the correlation between physical diagnosis and anatomy. These pictures alone make the book well worth while and are so recognized by the authors in their dedication when they say: "Without Dr. Fetterhoff's cooperation, cordial and self-effacing, our book would lack what is probably its most characteristic feature." E.W.B.

BOOKS RECEIVED

Greek Medicine in Rome. The Fitzpatrick Lectures on the History of Medicine Delivered at the Royal College of Physicians of London in 1909-1910. With other historical essays. The Right Hon. Sir T. Clifford Allbutt, K.C.B., M.A., M.D., F.R.C.P., F.R.S., Hon. F.R.C.P.I., Hon. M.D., Hon. LL.D., Hon. D.C.L., Hon. D.Sc., etc. 1921, 8°. 633 pages. Macmillan & Co., London.

Life and Times of Ambroise Paré, 1510-1590. With a New Translation of his Apology and an Account of his Journeys in Divers Places. By Francis R. Packard, M.D. With twenty-two text illustrations, twenty-seven full page plates and two maps of Paris of the 16th and 17th centuries. 1921, 8°. 297 pages. Paul B. Hoeber, New York.

The Oxford Medicine. By Various Authors. Edited by Henry A. Christians, A.M., M.D. & Sir James Mackenzie, M.D., F.R.C.P., LL.D., F.R.S. In six volumes. Illustrated. Volume IV, *Diseases of Lymphatic Tissue, Metabolism, Locomotory Apparatus, Industrial Disease and Infectious Diseases.* 1921, 8°. 938 pages. Oxford University Press. American Branch, New York.

History of the Pennsylvania Hospital Unit. (Base Hospital No. 10, U.S.A.) In the Great War. 1921. 8°. 253 pages. Paul B. Hoeber, New York.

A Laboratory Handbook for Dietetics. By Mary Swartz Rose, Ph.D. Revised edition. 1921. 8°. 156 pages. Macmillan Company, New York.

Woman's Hospital in the State of New York. Report of the Scientific Work of the Surgical Staff. Edited by George Gray

Ward, Jr., M.D., F.A.C.S. Volume III, 1920. 8°. 195 pages. C. V. Mosby Company, St. Louis, Mo.

Morris's Human Anatomy. A Complete Systematic Treatise by English and American Authors. Edited by C. M. Jackson, M.S., M.D. Sixth edition, revised and largely rewritten. Eleven hundred and sixty-four illustrations, five hundred and fifteen printed in colors. 1921. 4°. 1507 pages. P. Blakiston's Son & Co., Philadelphia.

American Child Hygiene Association. Transactions of the Eleventh Annual Meeting. 1921. 8°. 440 pages. Press of Franklin Printing Company, Baltimore, Md.

Columbia University in the City of New York. Studies from the Laboratories of the Department of Surgery. Volume III, 1918-1920. 8°.

Connecticut State Medical Society. Proceedings of the 129th Annual Convention. Editor Charles Williams Comfort, Jr. 1921. 8°. 265 pages. Published by the Society.

Ergebnisse der Chirurgie und Orthopädie. Herausgegeben von Erwin Payr und Hermann Küttner. Vierzehnter Band. Redigiert von H. Küttner. Mit 137 Textabbildungen. 1921. 8°. 956 S. Julius Springer, Berlin.

The Rockefeller Institute for Medical Research. Studies. Volume XXXVIII. 1921. 8°. 580 pages. The Rockefeller Institute for Medical Research, New York.

The Heart. Old and New Views. By H. L. Flint, M.D. With Illustrations. 1921. 8°. 177 pages. Paul B. Hoeber, New York.

BOOKS RECEIVED—Continued

- Syphilis and Its Treatment.* With Especial Reference to Syphilis of the Skin. By Wilfred S. Fox, M. A., M. D., B. C. (Cantab.), M. R. C. P. (London). With fifty-three illustrations, twenty-two in colour on fourteen plates and thirty-one in black and white on twenty-eight plates. 1921. 8°. 195 pages. Paul B. Hoeber, New York.
- Text-Book of Materia Medica for Nurses.* Compiled by Lavinia L. Dock. Seventh edition. Revised in accordance with the ninth decennial revision of the U. S. pharmacopeia. 1921. 12°. 315 pages. G. P. Putnam's Sons, New York and London.
- Modern Italian Surgery and Old Universities of Italy.* By Paolo De Vecchi, M. D. Foreword by George D. Stewart, M. D. With fifteen full page illustrations. 1921. 8°. 249 pages. Paul B. Hoeber, New York.
- The Microtome's Vade-Mecum.* A Handbook of the Methods of Microscopic Anatomy. By Arthur Bolles Lee, Hon. F. R. M. S. Eighth edition. Edited by J. Brontë Gatenby, B. A., B. Sc., D. Phil. (Oxon.), D. Sc. (Lond.), F. R. M. S. With the collaboration of W. M. Payliss, M. A., D. Sc. (Oxon.), F. R. S., F. R. M. S. [and others]. 1921. 8°. 594 pages. P. Blakiston's Son & Co., Philadelphia.
- The Diseases of Children.* By the Late Sir James Frederic Goodhart, Bart., M. D., LL. D., F. R. C. P. Eleventh edition. Edited by George Frederic Still, M. A., M. D., F. R. C. P. With 60 illustrations. 1921. 8°. 942 pages. Paul B. Hoeber, New York.
- The Principles and Practice of Medicine.* By the Late Sir William Osler, Bart., M. D., F. R. S. and Thomas McCrae, M. D. Ninth thoroughly revised edition. 1921. 8°. 1168 pages. D. Appleton and Company, New York and London.
- Diseases of the Skin.* By Richard L. Sutton, M. D. With nine hundred and sixty-nine illustrations, and eleven colored plates. Fourth edition, revised and enlarged. 1921. 8°. 1132 pages. C. V. Mosby Company, St. Louis.
- Studies in the Palaeopathology of Egypt.* By Sir Marc Armand Ruffer, Kt., C. M. G., M. D. Edited by Roy L. Moodie, Ph. D. 1921. 4°. 372 pages. The University of Chicago Press, Chicago, Illinois.
- The Lister Institute of Preventive Medicine.* Collected Papers. No. 15, 1918-19; No. 16, 1919-20. Part I. Bacteriological, Epidemiological, Pathological and Statistical Papers. Part II. Physiological, Zoological and Biochemical Papers. London.
- A Guide to Urinary Diseases.* By Adolphe Abrahams, O. B. E., M. D. (Cantab.) M. R. C. P., (Lond.) and A. Clifford Morrison, O. B. E., F. R. C. S. (Eng.). 1921. 8°. 120 pages. Longmans, Green & Co., New York. Edward Arnold & Co., London.
- Atlas for Electro-Diagnosis and Therapeutics.* By F. Miramond de Laroquette, M. D. Authorized Translation by Mary Gregson Cheetham. With Foreword by Robert Knox, M. D. 1921. 8°. 180 pages. Paul B. Hoeber, New York.
- The Blood Supply to the Heart in its Anatomical and Clinical Aspects.* By Louis Gross, M. D., C. M. With an Introduction by Horst Oertel. With twenty-nine full page plates and six text illustrations. 1921. 4°. 171 pages. Paul B. Hoeber, New York.
- A Manual of Selected Biochemical Methods as Applied to Urine, Blood and Gastric Analysis.* By Frank P. Underhill, Ph. D. 1921. 8°. 232 pages. John Wiley & Sons, Inc., New York.
- Surgical Diseases of Children.* A Modern Treatise on Pediatric Surgery. By Samuel W. Kelley, M. D., LL. D. Second edition, revised and enlarged; illustrated. 1914. 8°. 789 pages. E. B. Treat & Co., New York.
- A Pocket Surgery.* By Duncan C. L. Fitzwilliams, C. M. G., M. D., Ch. M., F. R. C. S. (Edin. and Eng.). 1921. 12°. 348 pages. Longmans, Green and Co., New York; Edward Arnold, London.
- Human Embryology and Morphology.* By Arthur Keith, M. D., F. R. S., LL. D. (Aberdeen), F. R. C. S. (Eng.) Fourth edition, revised and enlarged with nearly 500 illustrations. 1921. 8°. 491 pages. Longmans, Green and Co., New York; Edward Arnold, London.
- A Manual of Diseases of the Stomach.* By William MacLennan, M. B. With the Assistance of J. Salisbury Craig, M. B., Ch. B. 1921. 8°. 392 pages. Longmans, Green and Co., New York and London.
- The Life of Jacob Henle.* By Victor Robinson, M. D. 1921. 8°. 117 pages. Medical Life Company, New York.
- Clinical Surgical Diagnosis.* By F. de Quervain. [Third English edition.] Translated from the seventh edition by J. Snowman, M. D. With 731 illustrations and 7 plates. 1921. 8°. 914 pages. William Wood & Co., New York.
- The Oxford Medicine.* By various Authors. Edited by Henry A. Christian, A. M., M. D., and Sir James Mackenzie, M. D., F. R. C. P., LL. D., F. R. S. Volume V. *Infectious Diseases (Cont'd) and Diseases Due to Animal Parasites.* Volume VI. *Diseases of the Central Nervous System.* Under the Editorial Supervision of Sir James Purves Stewart, K. C. M. G., C. B., M. D., F. R. C. P., 1921. 4°. Oxford University Press, American Branch, New York.
- Oxford Medical Publications.* Publishers: Henry Frowde, London; Hodder & Stoughton, London. The following 5 volumes:
- The Early Diagnosis of the Acute Abdomen.* By Zachary Cope, B. A., M. D., M. S., Lond., F. R. C. S. Eng. 1921. 8°. 223 pages.
- The Care of Eye Cases.* A Manual for the Nurse, Practitioner and Student. By Robert Henry Elliott, M. D., B. S. (Lond.), Sc. D. (Edin.), F. R. C. S. (Eng.). With 135 illustrations. 1921. 8°. 172 pages.
- Heart Disease and Pregnancy.* By Sir James Mackenzie, M. D., F. R. C. P., LL. D., Edinburgh and Aberdeen. F. R. S., F. R. C. P. I. 1921. 8°. 138 pages.
- Obstetrics and Gynaecology.* Edited by John S. Fairbairn, M. A., B. M., B. Ch. (Oxon), F. R. C. P. (Lond.), F. R. C. S. (Eng.) 1921. 4°. 950 pages.
- The Anatomy of the Human Orbit and Accessory Organs of Vision.* By S. Ernest Whitnall, M. A., M. D., B. Ch. (Oxon.), M. R. C. S., L. R. C. P. (Lond.) Illustrated largely by photographs of actual dissections. 1921. 8°. 428 pages.

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THE EVOLUTION OF HUMAN RACES IN THE LIGHT OF THE HORMONE THEORY*

By Prof. SIR ARTHUR KEITH

(Conservator of the Museum and Hunterian Professor, Royal College of Surgeons, London, Eng.)

There is no need for a Herter Lecturer to apologise to Alumni and students of Johns Hopkins University for choosing a subject which has apparently no direct bearing on medical practice. No University has perceived more clearly than Johns Hopkins that rational medical practice must be based on a complete knowledge of the normal machinery of the animal and human body and has, therefore, in the past welcomed every enquiry which was likely to broaden the basis of this knowledge. When we set out to discover how the races of mankind have arisen, we have to make a full demand on all we know concerning the factors which regulate the growth of the

human body—particularly on the action of the glands of internal secretion. Much of what the world knows concerning these glands it has learned from Johns Hopkins University—from Howell, Cushing, Goetsch, Abel, Barker, MacCallum and many others. In these lectures I hope to return, with some degree of interest, the preliminary outfit which the anthropologist borrowed from the clinician and pathologist.

DARWIN, HUNTER AND THE HORMONE THEORY

I can best introduce the problems to be discussed in these lectures by carrying my audience to Darwin's home, near the village of Down, situated in the chalk uplands to the south of London, where, in the year 1870, the great observer was applying his machinery of evolution to the

* Abstract of Lecture I under the Herter Foundation delivered before the Johns Hopkins University on October 5, 1921.

"Descent of Man." After he had made use of all the natural agencies at his command, the action of selection, the supposed effects of use and disuse, the manner in which one part of the body is correlated with that of another part, he had to declare that none of these could explain how the Negro had come by one set of characteristic features, the Mongol by another set and the European or Caucasian by a third. The missing agency which Darwin was in search of has been discovered accidentally by medical men working on disorders of nutrition and growth in the out-patient departments and wards of public hospitals. It was working under such circumstances that clinicians came to realize that there is placed in the human, as in the animal body, a group of glands, which, through the formation of chemical substances—or Hormones as Starling named them in 1904—determine the individual and racial characters of every man, woman and child. It is a matter worthy of remark that not only Darwin but also his great predecessor, John Hunter, stood on the verge of this discovery—and yet both were sidetracked. John Hunter successfully transplanted the testes from one fowl to another; he grafted the cock's spur on the hen's leg, and the hen's spur on the cock's leg; his earliest investigation was to show that the human testis which failed to descend was arrested because of imperfect development; no one ever realized more clearly than Hunter did that the growth of all these features of the body, which he named "secondary sexual," depends on the presence of healthy sexual glands. Darwin also realized this to the full, but he concentrated his attention, not on how sexual characters are produced but on how they may be selected and perpetuated once they have come into existence. In the history of discovery we learn as much from the failures of brooding geniuses as from their successes. Both Hunter and Darwin had the misfortune to work ahead of the physiological knowledge of their time.

THE FEATURES OF NEANDERTHAL MAN AND OF THE ACROMEGALIC CONTRASTED

Soon after 1908, when the Council of the Royal College of Surgeons of England placed me in charge of the Museum which a long line of Conservators had built up round Hunter's glorious nucleus, I had to rearrange the skulls of ancient and modern human races. In one section were two specimens of historical interest; one was a cast of the Neanderthal calvarium which Shaaflhausen had sent to Huxley in 1860 and on which the latter had based his statement that Neanderthal man was the most primitive, most ape-like and most ancient type of humanity known to him. The other specimen was a remarkable modern skull which led Dr. Barnard Davis, the British craniologist, flatly to contradict Huxley by asserting that Neanderthal characteristics could still be found in the population of Europe. I noted with interest that the pituitary fossa in the Barnard Davis skull was double

the ordinary size, that all the characteristics of a regulated acromegaly were present and that it was these acromegalic traits which gave the apparent resemblance to the Neanderthal type. At the date of which I write there was ample evidence to prove that a disordered enlargement of the pituitary was directly related to the appearance of acromegalic traits and that the effects could be best accounted for by regarding the pituitary as a centre in which growth-hormones were elaborated. The query naturally arose in my mind, as it did in that of others: Can the pituitary be concerned in the evolution of human races?

At the time I commenced this investigation an important truth was dawning on the minds of European anthropologists. The late Professor Gustav Schwalbe of Strassburg, strongly backed by Dr. Adloff of Berlin, led a movement which completely altered the then prevailing conception of Neanderthal man; Schwalbe held that he must be regarded, not as the Pleistocene ancestor of modern Europeans, but as a separate and extinct species of humanity. With the establishment of Schwalbe's position, we had to alter our mental vista of man's past. All the existing races of mankind are members of a single species but as we go into the far past we find not only separate species of humanity, but separate genera. We have to base our conception of the conditions under which mankind was evolved not on what we see in the modern world of man, but on what we can see now among man's nearest allies—the anthropoid apes. These we find broken up into diverse genera and species, confined to definite localities. All discoveries of recent years justify the belief that in the remote past mankind was represented by numerous local genera and species and that the machinery of evolution must have been both active and effective. The nature of this machinery is not beyond recall; we may safely presume that it may be studied now in living groups of anthropoid apes—amongst gorillas, chimpanzees and orangs. We shall find among anthropoid apes the same hormonal mechanism which shaped the ancient and modern racial types of mankind.

Here, too, before following the straight thread of my lecture, I may mention another line of evidence which has influenced the direction of my investigations. We are apt to think of evolution as a process of the past and that mankind has now come to an evolutionary standstill—so far as racial differentiation is concerned. When we look at the distribution of the chief types of humanity as seen in the modern world it is no longer possible to share in this belief. We cannot explain why the Mongolian type was formerly confined to one region of the earth, the negro to another and the European to a third, or why intermediate types link them together, unless we proceed on the hypothesis that evolution has been and is now at work. In every continent we find human races at all stages of differentiation—from an

incipient state such as is exemplified by the British Anglo-Saxon and Celt, where the degree of physical differentiation is slight, up to a complete stage, such as represented by Negro and white man where the degree of physical differentiation is unmistakable. The quest we set out on is the discovery of the physiological machinery concerned in the differentiation of mankind into its various physical types.

ACROMEGALY IS A TRUE DISORDER OF GROWTH

For the purpose of our search no condition is so instructive as that known to clinicians as Acromegaly. In a short period of years after the onset of this disorder, a new and distinctive type of being is produced which the clinician recognizes at a glance as acromegalic. All the bodily structures and characters involved in the change are just those which the anthropologist knows to be concerned in the differentiation of human races. The texture and frequently the tint of the skin is changed; the growth and distribution of hair alters, the nose, lips, jaws, cranium and thorax undergo characteristic transformations; stature and the proportions of the body and limbs become modified. To study and estimate the degree and nature of these acromegalic changes we must apply the same exact methods as anthropologists employ in the study of human races.

Ten years ago I published the results of a craniological examination of acromegalic skulls¹ and showed the general nature of the changes which had occurred in them. At that time I had not sufficiently appreciated the complexity of the processes involved in the growth of the normal cranium, for, to provide room for the growing brain, new bone has to be laid down at varying rates along certain sutural lines, while along other lines it is being absorbed. No less elaborate is the growth mechanism of the face, for, as the teeth erupt, additional bone is interpolated along certain sutural lines and deposited on certain areas while a process of absorption is taking place along other lines and areas. Each bone of the cranium and face represents a separate growth element and it is only by studying the changes which occur in each element that we come by a precise knowledge of how the face of a boy of five years of age is transformed into the face of an adult man. The result of study along these lines on additional acromegalic material has been to show that the differences between a normal and an acromegalic skull are exactly of the same nature as those seen when we compare the skull of a boy in his thirteenth year with one of an adult man. Beyond any doubt, with the onset of acromegaly a true growth process is reawakened and that reawakening is a continuation of the normal process which should cease when the adult stage is reached. The disorderly enlargement of the pituitary is directly related to the resurrection of the process of growth.

¹ An Inquiry into the Nature of the Skeletal changes in Acromegaly. *Lancet*, April 15, 1911.

ACROMEGALIC CHANGES HAVE A FUNCTIONAL SIGNIFICANCE

It is only when we realize that the size and shape of the skull are determined by the functional purposes which it has to serve and by the manner in which it serves them, that we really profit by craniological enquiry. In the first place the skull has to serve as a brain carrier, providing a cavity with walls so fashioned that additional accommodation can be easily and economically provided for the growing brain. In the second place, the skull has to serve as a carrier of sense organs—the eye, nose and ear. In the third place, it has to provide the whole of the bony scaffolding concerned in mastication, and which also answers, in a secondary degree, to the needs of speech and respiration and deglutition. In the fourth place it has to serve as a complex mobile lever on which the muscles of the neck act.

Let us look for a moment at the alterations undergone by acromegalic skulls in respect to these four functions. Apparently the brain increases in bulk and the cranial cavity in size; at least the mean cranial capacity of the acromegalic skulls at my disposal is distinctly above the average for normal skulls. As a sense carrier there is no change, save as regards the nose. But as regards its third or masticatory function, very remarkable and instructive changes occur. The area on the side of the skull from which the temporal muscle arises, becomes greatly extended, particularly in a forward direction. In the forward extension the frontal bone undergoes a transformation involving changes along the coronal suture, the throwing out of great supra-orbital bulwarks which serve as fulcra of origin for the temporal muscle. The transformation is usually facilitated by an enormous extension of the frontal air sinuses. The origin for the masseter muscle—the zygomatic arch—is strengthened, elongated and lowered in position. The areas and processes from which the pterygoid muscles arise, are also strengthened and extended. The ascending ramus of the mandible, which has to provide an area of insertion for those muscles, elongates greatly and sometimes increases in width as well. All parts of the body of the mandible become the sites of true growth; the alveolar margin, carrying the tooth-sockets, is remodelled and extended. The chin and the lower mandibular border are greatly augmented—strengthening and extending the bony scaffolding on which the floor of the mouth is set. All parts of the upper jaw—with one exception—share in the general enlargement of the masticatory system. The exception is the area carrying the alveolar margin and hard palate; these parts, we shall see anon—may not suffer a perceptible degree of change.

Now the parts just named—the chin, the size and form of the mandible, the prominence of the cheek-bones, the size and projection of the ascending nasal process of the

upper jaw, the degree of development of the supra-orbital ridges—are all of them immediately concerned in giving the face its individual, as well as its racial, characteristics. All of them are linked together to serve in one function—mastication. The pituitary acts not in an anatomical but in a physiological manner; it can somehow stimulate and correlate the growth of all the anatomical elements concerned in a single function. We shall see presently that other hormones, besides those arising in the pituitary, can and do influence the growth of the face. It is sufficient for the meantime to be certain that the characterization of the face—the chief signboard of race—is under the control of a hormone system.

Further evidence in favour of the view that the growth functions of the pituitary are grouped on a physiological—not an anatomical—basis is seen when we study the adaptations undergone by the skull to serve as a lever. To that area of the skull to which the muscles of the neck are attached we may give the name of “nuchal platform”; it is fashioned out of cartilage bone, comprising the whole of the cartilaginous part of the occipital bone and the mastoid part of the temporal bone. In acromegaly the nuchal platform has its area greatly enlarged; all its bounding and intercrossing muscular ridges—comprising the mastoid processes and occipital ridges—are enormously strengthened. The need for some mechanism to correlate the growth of the masticatory bony scaffolding and nuchal platform is made very evident in the skulls of anthropoid apes. The gorilla exerts the strength of its massive body through its huge jaws; hence in this animal a supreme development of the masticatory scaffolding is correlated with an enormous expansion of the nuchal platform. In the chimpanzee, a near relative of the gorilla, the jaws are reduced and so is the nuchal platform. The difference between Neanderthal man and modern man—in respect of jaw and neck—corresponds in degree to that seen between gorilla and chimpanzee. As a boy's jaws grow, his neck enlarges; he continues to take a bigger size in collars as each new tooth erupts. In women the head is poised on a slender neck; their maxillary and nuchal development represent an adolescent stage of the male. In these instances, we see the pituitary acting on a series of anatomical elements moulding and adapting them to a single physiological end. Nuchal characters also serve to differentiate races. It is a remarkable fact that the most extensive facial and nuchal development is found amongst Europeans.

ALL SYSTEMS OF THE BODY ARE AFFECTED

IN ACROMEGALY

Too much stress cannot be laid on the fact that in acromegaly all the tissue systems of the body are involved. In every case of acromegaly which has received a complete examination, it has been found that not only are

the skeletal and cutaneous tissues affected but so too are the circulatory, the pulmonary, the alimentary, renal and connective tissue systems. All of them are involved in overgrowth with the exceptions of the sexual system, which undergoes atrophy, and the nervous system, regarding which our evidence is incomplete. Nor can too much emphasis be laid on another fact that in the later stages of the disease—all of these systems suffer degeneration and atrophy. Some years ago the Museum of the Royal College of Surgeons obtained a subject of this disease—a man who had begun to show symptoms of acromegaly at the age of twenty-four and had died twenty-five years after the onset, the disease having been steadily progressive. Preparations, naked-eye and microscopic, were made from all the systems and organs of this individual and are now preserved in the Museum of the College. Every cell of the body of this subject was found to have suffered in the general disorder of growth. For example, the muscles of mastication had not merely undergone a normal degree of hypertrophy; they had undergone a real process of growth—the individual fibres having increased in length, diameter and number. This was also true of all the muscles of the tongue and mouth. The temporal and facial arteries were abnormally large and their muscular coats greatly increased, but, as was the case throughout the whole arterial system, the inner coat was thickened, it encroached on the arterial lumen. From these facts one infers that the pituitary acts upon all the structural elements which are comprised within a functional unit and somehow keeps their growth in unison.

THE NATURE OF THE DISORDER KNOWN AS ACROMEGALY

So far I have merely put forward the evidence in support of the view that in Acromegaly we have exposed for us part of the growth-machinery concerned in the differentiation of racial characteristics. The pituitary and substances formed within it are essential parts of the machinery. Before we proceed further it is necessary to ask the question: Is Acromegaly the result of a breakdown of one of the growth mechanisms of the normal body? I think this question must be answered in the affirmative. It is a breakdown of the body's chief adaptational mechanism—the mechanism which “makes the back equal to the burden it has to bear.” That a mechanism of this nature must be present in the body, becomes apparent, I think, if one will but mark what happens in the various systems of the body when a man or animal is placed under training. The muscles are the structures chiefly concerned but it would be useless for them to undergo a hypertrophy unless they had some means of calling forth a corresponding growth reaction from heart and vessels so as to be supplied with an increased amount of blood, from the lungs, to meet their increased need of oxygen; from the alimentary system, so as to be supplied with increased energy-material; from the kidneys, so as to rid them of their increased refuse

products, from the skeletal and articular and ligamentous systems, so that they become fit to withstand the increased stresses produced by hypertrophied muscles. These effects cannot be produced in a merely mechanical way; we must postulate an organized system of some kind and the one which answers all the circumstances of the case is that of hormones. In Acromegaly we find a disorderly manifestation of all the results which follow increased use of muscles—bones strengthen, particularly their muscular ridges and impressions; the joint-surfaces enlarge, the ligaments are thickened, the heart, arteries and veins hypertrophy, the lungs and chest undergo a true growth; the apparatus of mastication and the alimentary canal become hypertrophied, renal substance is increased. If we suppose that this adaptational mechan-

ism, which makes hypertrophy answer to increased action, were to be set in motion, not through the normal stimulus of muscular activity but through some condition arising in the pituitary itself, then we can understand why it is that the human body should be subject to such a disorder as Acromegaly.

One other fact supports the theory here put forward regarding the nature of Acromegaly. Why should the hands and feet be the parts of the body which suffer first and most in Acromegaly? If my explanation is the true one, then the feet and hands should be the most susceptible, for nothing is more certain than that hard manual labour will produce marked growth responses in the hands and feet.

THE PHARMACOLOGICAL ACTION OF ADRENALIN ON THE SPHINCTER PYLORI OF THE FOETUS

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AND

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Pirie's † recent suggestion that hypertrophic stenosis of the pylorus in newly born infants may be due to fetal hyperadrenia makes it necessary to view that condition from an entirely new angle.

The development of hypertrophic stenosis according to Pirie comes about as follows:— (1) Reflex stimulation of the adrenal medulla from some internal or external receptor, *e. g.*, the preputial nerve endings, is followed by (2) the discharge of an increased amount of adrenalin into the blood stream.‡ (3) This increased adrenal discharge stimulates the splanchnic and causes, according to whether it is constant or intermittent, either increased tone or spasmodic contraction of the alimentary sphincters (ileocolic and pyloric) because of the so-called reverse innervation of these muscle groups. Hypertrophy of the sphincter pylori follows because of this spasm or increased tone, and persists into post-fœtal life. The cause of stimulation disappears or is persistent only as some minor defect (phimosis) which is not usually recognized as causally associated with the graver pathological result. It may, however, be sufficient to maintain

the severity of the morbid condition for which the infant comes under treatment.

Pirie cited cases of babies suffering from hypertrophic stenosis who recovered after circumcision and mentioned the autopsy of a child dead from hypertrophic stenosis at which gross adrenal lesions were found to support this attractive hypothesis. It is obvious that this hypothesis is dependent on the so-called "reversed innervation" of the gastro-intestinal sphincters. In other words, the statement must be accepted that while the augmentor fibres supplying the gastro-intestinal musculature in general reach the digestive tract from the vagus nerve and the inhibitors from the splanchnic, the muscles of the pyloric and ileo-colic sphincters are augmented through the splanchnic and depressed by way of the vagus. Stimulation of the splanchnic nerves (peripheral), according to this theory, results in relaxation of the entire gastro-intestinal musculature except the ileo-colic and pyloric sphincters which are said to be thrown into a state of increased tonus by the impulse following splanchnic excitation.

Almost nothing is known about the behavior of the intestine during intra-uterine life.

It has been generally accepted that peristalsis occurs before birth because lanugo hairs are found in the first meconium. These hairs could only have reached the large intestine by passing through the whole gastro-intestinal tract after having been swallowed with amniotic

† Pirie, G. R., *Lancet*, September 20, 1919.

‡ In the *British Medical Journal* of November 26, 1921, (p. 891) Gray and Reynolds have suggested that the hypersecretion of adrenalin may be maternal as well as fetal.

fluid into which they had been cast off. The general opinion regarding ante-natal intestinal movement has been that peristalsis is feeble and infrequent until just before the birth of the fetus. Yanese,* indeed, found that in human fetuses he was able to produce peristalsis and local contractions as early as the ninth week of intra-uterine life. Before this date, when the myenteric plexuses and the longitudinal muscle coat begin to develop, this author claimed to have been able to produce neither peristaltic movements nor local contractions by direct stimulation. He concluded, therefore, that peristaltic movements are of neurogenic rather than myogenic origin.

In the course of a study of the physiology of the gastro-intestinal tract in the fetus we have observed the action of adrenalin solutions on the musculature of the stomach and intestine, and since the appearance of the hyperadrenia theory of pyloric stenosis, it has seemed worth while to record the reaction of the fetal sphincter pylori to solutions of adrenalin. Solutions of adrenalin were tested on ring and strip preparations of isolated sphincter pylori and on preparations of the pylorus *in situ*.

Isolated preparations of embryonic pyloric muscle show very characteristic regular spontaneous contractions when kept in oxygenated warm Ringer solution. The graph made by such an isolated ring is shown below (Fig. No. 1). Contractions usually start two or three minutes after the preparation is set up and increase steadily and rapidly in strength until a maximum contraction is reached, which may be maintained for two or three hours. While the contractions are usually singularly rhythmic, the rhythm maintained may be multiple (in groups of two or three beats, Fig. No. 2) and occasionally, in chilled or shocked preparations, the contractions are irregular in rate and force. The character of the curve made by these muscular contractions is strikingly uniform—a rapid contraction indicated by a steep, almost vertical ascending limb, and a rapid initial relaxation slowing to its completion, so that the descending limb of the curve blends gently with the horizontal. Ascent or relaxation may be interrupted by secondary contractions which produce ana- or cata-crotic notches preceding the relaxation of the muscle, or the curve may be topped by a smooth or serrated plateau; double contractions may occur which are followed by a contraction wave of extra height, or by a compensatory pause, a phenomenon which we shall discuss in more detail later.

Now if a solution of adrenalin be instilled into the Ringer bath in which the strip of muscle is working, a pronounced change in the rhythm and force of the con-

traction takes place which is characteristic and constant for a given dose of the drug. If a small dose of the salt is given (0.0005 mgm. in 50 c.c. of the bath solution), the interval between the contractions is lengthened immediately so that three or four times the usual period may intervene between contractions without any departure of the lever from the base line (Fig. 3b). When the muscle does contract, however, it may do so with somewhat greater force, so that the resulting curve of the contraction wave is very slightly higher than the curve of the contractions immediately preceding the instillation of the adrenalin solution. On the other hand, after a slightly larger dose, the amplitude of the contraction may be diminished, the interval remaining the same, or both amplitude and frequency of contraction may diminish. When the amplitude of the contraction is very much diminished, the return to normal contraction force may be gradual (Fig. 3a). This is maintained until the preparation is washed out or the drug is destroyed by oxidation. This decreased frequency of contraction is more pronounced as larger doses of the drug are given until, following the exhibition of massive amounts (0.3 mgm. in 50 c.c.), the muscle may remain completely paralyzed for half an hour or more, even though the specimen has been twice or three times washed. Return of contractility to such a paralyzed muscle is like the onset of contractions in a fresh, shocked, preparation. The muscle contracts at first to a hardly recordable extent and gradually progressively, minute by minute, the contraction attains its full strength (Fig. 4). The action of adrenalin on pyloric strips is quite the same.

The description of the increase in the size of the contraction following the administration of small doses of adrenalin might make one feel that, besides the depressor action of the drug, some pressor action was present also which increased the contraction of the muscle, but a study of the graphs made from untreated preparations shows that an increase in the strength of contraction is seen following any pause in the course of the spontaneous rhythmic muscular movements or lengthened interval between contractions. It will be seen, therefore, that this apparent increase in contraction is analogous to the heightened response of the cardiac muscle after the removal of the inhibition caused by vagus stimulation.

The above account of the results of splanchnic stimulation holds good not only in the pig fetus, on which most of our studies have been made, but also in all other mammals which we have studied, including man. Since pyloric stenosis occurs only in man, as far as we at present know, an examination of human fetal material is necessary in order to exclude the possibility of special peculiarities. This is especially so since human tissue, at least in one

* Yanese, T.: Arch. ges. Physiol., LXVII, 345-383.

instance, is said to vary from that of other mammals in its response to chemical stimulation (see Barbour's description of the response of the human coronary artery to adrenalin). It may be well, therefore, in this connection to give a protocol of one of the experiments on the pylorus of a human fetus.

The child R. M., female, prematurely born in the sixth month, weight 907 gms., admitted in the eleventh hour of extra-uterine life. The child was said to have grown progressively weaker since birth.

Death at 8.30 A. M., the day following admission.

At 10 P. M. the stomach was opened and found filled with a slightly alkaline fluid consisting of the milk feeding given two hours before death. The milk showed no evidence of coagulation.

The pylorus was normal to inspection. It was cut into strips one of which was immediately suspended in 50 c.c. of oxygenated Tyrode's solution at 39° C. and attached to a light lever.

Strip No. 1. Spontaneous contraction of the muscular strip started immediately and continued with experimentally induced variation until 11.20 P. M., when the preparation was discarded. The instillation into the bath of 0.1 c.c. of a 1 : 100,000 solution of adrenalin chloride (P.D.) (a concentration of 1 : 50,000,000) was followed by no other change in the contraction than a slight slowing of the contraction rate. The instillation of double the amount of the drug was equally unproductive of appreciable effect. Following the instillation of 1 c.c. of 1 : 100,000 solution of the drug, however, (1 : 5,000,000) the amplitude of the contraction curve was considerably diminished and the exhibition of 1 c.c. of a 1 : 10,000 solution (1 : 500,000 concentration) caused a relaxation of muscular tone and diminution of contraction amplitude to approximately one-fifth of the normal value, a condition of affairs which persisted until, just before removal of the strip from the bath, stimulation with 0.1 mgm. of histamine restored approximately the pre-experimental muscular vigor (Fig. 5).

Strip No. 2, four hours and 20 minutes after death, 2 hours and 50 minutes after removal from the cadaver, gave an equivalent reaction to that given by preparation I.

Strip No. 2, studied at 3.30 P. M. on the day following death under the same conditions described above, gave only feeble contractions which were marked by inhibited adrenaline chloride 1 : 50,000,000 and which entirely ceased under the influence of the same drug 1 : 5,000,000. This strip was kept at refrigerator temperature in Ringer's solution for 31 hours after death.

It will be seen from the above experiment that the reaction of the human foetal pylorus differs in no respect from the response to adrenalin given by the pyloric musculature of fetuses of other mammalian species. This response is in the nature of relaxation and inhibition rather than of stimulation and augmentation.

We have been able to confirm the results given by ring

and strip preparations, isolated from the fetal body and maintained *in vitro*, by experiments in which the pylorus was left *in situ* and the adrenalin was exhibited to the pylorus with the blood and nerve supply uninterrupted.

Increased secretion of adrenalin it would seem, could hardly result in anything else than a decrease in the tone of the pyloric muscle, and intermittent discharges of adrenalin, instead of inducing periodic pyloric spasm, would rather result in periods of relaxation of the gastro-intestinal motor mechanism.

Furthermore, since the action of adrenalin on the muscle of the gastro-intestinal tract depends on stimulation of the endings of the splanchnic, reversal of valvular innervation, if it exists in the adult intestine, must be a phenomenon confined to post-fetal life.

It is interesting to note that in the pig fetus, even in those with a C. R. length of only 6 cm., it is possible to see definite peristalsis when the gut is exposed after delivery from the uterus. Foetal peristaltic movements follow the "law of intestine" as laid down for the adult animal, bear the same relation to the Tonus rings and are affected in the same way by stimulation. Contractions of segmentation occur and are more readily seen than in the adult gastro-intestinal tract and, in brief, intestinal motor mechanism has the potentiality to carry out the movements described as characteristic for the gastro-intestinal tube in post-embryonic life.

FIG. 1.—Spontaneous contractions of an isolated ring of embryonic pyloric muscle (pig).

FIG. 2.—Spontaneous contractions of a ring of pyloric muscle from a pig embryo, to show compound contractions and multiple rhythm in a cooled preparation. Temperature of the bath 36° C.

FIG. 3.—a and b show the effects of small doses of adrenalin chloride on the contractions of rings of embryonic pyloric muscle (pig) suspended in 50 c.c. of Ringer-Locke solution. While the contraction rate is much slower after very many doses of adrenalin, the tone of the muscle remains unchanged. Recovery may be sudden or gradual. During the period of recovery the amplitude of the contractions may return to normal or slightly above, while the frequency is still diminished. The immediate effect of the drug is the diminution of both amplitude and frequency of contraction.

FIG. 4.—Shows complete cessation of contractions of a ring of pyloric muscle of a pig embryo after 0.3 mgm. of adrenalin chloride was added to a bath of 50 c.c. Ringer-Locke solution.

FIG. 5.—Curve showing the effect of adrenalin chloride on a preparation of the pyloric muscle of a human fetus which contracted spontaneously in 50 c.c. of Ringer-Locke solution. This curve shows that the tone of the muscle was impaired by the drug as well as the frequency and amplitude of the contractions.

FIG. 1.

Spontaneous contraction of a ring of pyloric muscle. Pig fetus C. R. 115 mm. Ringer-Locke solution.

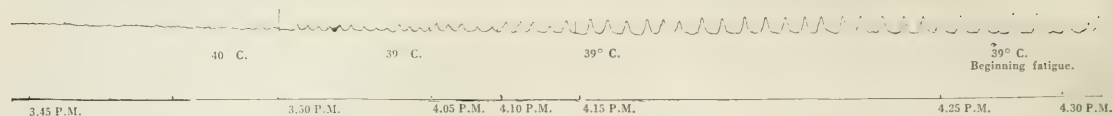


FIG. 2.

Compound contractions and multiple rhythm of a ring of pyloric muscle induced by cooling. Pig fetus C. R. 146.1 mm.

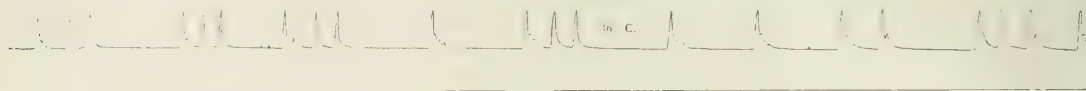


FIG. 3a.

Pylorus of a pig fetus. C. R. 157 mm. in 20 c.cm. of Ringer-Locke solution.

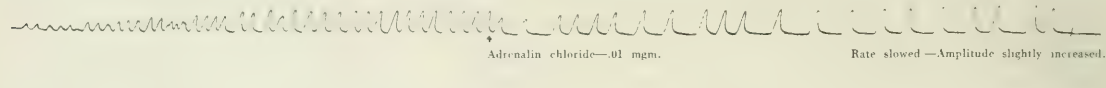


FIG. 3b.

Effect of Adrenaline Chloride on pylorus of a pig embryo. C. R. 171 mm. Ringer-Locke. 38° C.

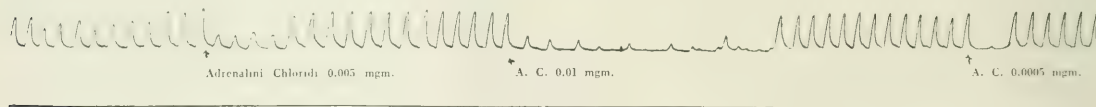


FIG. 4.

Effect of adrenaline chloride on the spontaneous contractions of the pylorus. Pig embryo C. R. 155 mm. Ringer-Locke solution. 20 c.cm.

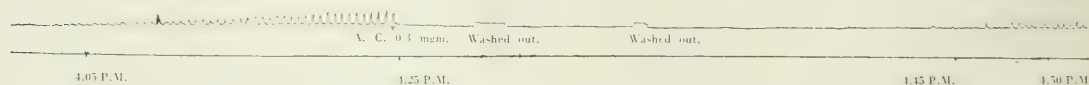
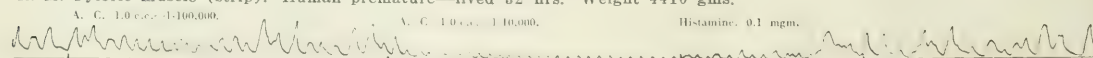


FIG. 5

R. M. Pyloric muscle (strip). Human premature—lived 32 hrs. Weight 4410 gms.



OCCURRENCE OF ANAEMIA IN RATS ON DEFICIENT DIETS

By WILLIAM M. HAPP

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Introduction. Numerous attempts have been made to produce anaemia in animals by means of diets poor in iron. In these experiments attempts have often been made to ascertain whether inorganic iron given by mouth causes the regeneration of the blood in anaemia. Meyer¹ summarized the literature on this subject in 1906.

Hall² fed white mice on the following diets:

1. Casein	37.8
Starch	28.3
Fat	30.1
Salts*	3.7
2. Casein	20.0
Butter	3.34
Fat	11.66
Cellulose	0.7
Starch	60.5
Salts	3.7

He found that the total iron content of the ash of the mice kept for three weeks on these diets decreased 40 per cent. The feeding of iron by mouth increased the number of red blood cells and haemoglobin. The ingredients of these diets were purified foodstuffs and relatively free from iron. The nature of the fat used was not stated. The animals lost rapidly in weight and lived only four weeks on diet No. 1 and six weeks on diet No. 2. No water soluble B was supplied in these diets.

Schmidt³ was not able to produce anaemia in the first generation of white mice fed on a diet poor in iron. The blood of their young, however, showed a reduction of haemoglobin and red blood cells, with poikilocytosis, anisocytosis and polychromasia. When iron was added to the diet, the blood became normal. Enlargement of the spleen was noted in these anaemic mice.

Other authors, Kunkel,⁴ Cloetta,⁵ Abderhalden,⁶ Müller,⁷ Tartakowsky,⁸ and others, fed animals on diets of milk, milk and bread, and milk and rice, to study the regeneration of the blood following bleeding and the effects of the administration of inorganic iron. These experiments showed that a reduction in the amount of iron bound in the tissues (reserve iron) and in the circulating blood (circulating iron) resulted in animals fed on such diets. The depletion could be made up by feeding iron by mouth either in inorganic or organic combination. They showed further that the reserve iron was depleted before the amount of iron circulating in the blood as haemoglobin was diminished. There have

been few studies other than those regarding the haemoglobin content of the blood of animals on deficient diets.

It is a fact that the prolonged feeding of milk alone to an infant may be followed by the appearance of a secondary anaemia. Because of the work of Bunge⁹ and Abderhalden⁶ this anaemia has been attributed to a depletion of the reserve store of iron with which these infants are born, and a consequent reduction in the circulating iron (haemoglobin). Again, there are anaemias associated with rickets in infancy in which a peculiar symptom complex is present, associated with various qualitative and quantitative changes in the blood picture. Children with these anaemias usually have an enlargement of the spleen, which is occasionally extreme, and a slight enlargement of the liver and lymph nodes, and a secondary anaemia with or without leucocytosis. There is often a lymphocytosis, and immature cells of both red and white blood cell series are found in the circulating blood (normoblasts, megaloblasts, myelocytes, pathological lymphocytes, etc.). This clinical picture has received various titles, such as "von Jaksch's anaemia," "anaemia pseudoleukämica infantum" and "splenic anaemia of infants." It is in all essential respects a secondary anaemia. The frequency of its association with rickets has been emphasized by practically every author on the subject (see review by Evans and Happ).¹⁰ The cause of this anaemia has not been established. Anaemia of this type appears to depend upon more factors than does the simple secondary anaemia which results from a diet low in iron, such as a prolonged milk diet.

On account of the frequency of anaemia in infants with rickets the question arose as to the existence of anaemia in rats made rachitic by faulty diets. Through the kindness and co-operation of Dr. McCollum, Miss Simmonds and Doctors Shipley and Park, I was able to study the blood of rats on various faulty diets. These authors have found that if growing rats are placed on diets in which certain fats, butter fat, cod-liver oil, etc., are absent, or present only in small quantity, and in which there is a certain disproportion between the amounts of calcium and phosphorus, lesions of the bones result which are analogous to the lesions of the bones of young children with rickets.¹¹

The result of the examination of the blood of rats on various faulty diets is given, together with the diet employed in each instance.

Technic. Klineberger and Carl,¹² and later Powdermaker,¹³ found that the number of cells in blood obtained from the tails of rats was subject to great variation. For this reason the following procedure was employed in obtaining blood for

* Composition of salt mixture.

K ₂ CO ₃	0.97
NaCl	0.7
CaHPO ₄	1.8
MgCl ₂	0.18

counting. The rat was lightly anesthetized and laid on a board, ventral side up. The hair over the thigh was cut close. The femoral (superficial) vein, which lies just beneath the skin, was exposed. This vein is quite prominent, especially in albino rats. It was cut with sharp scissors. In this way sufficient blood could be obtained with insignificant trauma for a red and white blood cell count, hæmoglobin determination, a cresyl blue preparation for reticulated red blood cell count, and smears for a differential blood count. As a rule, the flow of blood stopped promptly, and if the animal was to be kept, a drop of collodion was placed on the wound. The counts given in this paper, however, are the results of the first examination, unless otherwise stated. No animal was bled previous to the making of the blood count, the results of which are given. The red and white blood cell counts were done in the ordinary manner, Türk's and Hayem's solutions being used for diluent, and the blood was counted in a Karl Zeiss hæmocytometer with Neubauer ruling. The hæmoglobin determinations were made with the Sahli apparatus. The hæmoglobinometer standard was so prepared that it gave a reading of 95 per cent for blood from the normal human adult. A reading of 95 per cent indicated approximately 14.25 grams of hæmoglobin per 100 c.c. of blood. The blood films for differential counts and for the counting of the reticulated red blood cells were made by the following technique: A clean glass pipette of small bore or a white blood cell pipette was rinsed with a saturated solution of sodium oxalate, the fluid withdrawn and the inside of the pipette thoroughly dried. Several drops of blood from the incised thigh vein were drawn up into the pipette and blown in and out several times on a watch-glass. In this manner the blood was kept from clotting. A film was made from this oxalated blood in the usual manner. These films were stained, when dry, with Wilson's modification of Romanowsky's stain, mounted on a slide, a differential count of the white blood cells was made and the nature of the red blood cells noted. Then a drop of the oxalated blood was drawn into the pipette together with a drop of 1 per cent cresyl blue in normal salt solution. The blood and the stain were well mixed by blowing the contents on a watch-glass. A drop of this mixture was then blown on a cover-slip, films were made and stained with Wilson's modification of Romanowsky's stain, dried and mounted. By this method a preparation was obtained which showed beautifully the reticulum in the red blood cells. These preparations were permanent. Films kept for one year in this laboratory still show the reticular stain of the red blood cells. The percentage of reticulated cells was determined by counting a certain number of red blood cells, 500 or 1000, and recording the number of cells showing reticulum.

Cages. In these experiments three kinds of cages were employed. They are designated as

1. Galvanized iron cages. The iron wire was well insulated by galvanized metal.

2. Wood and iron cages. These are standard cages used in Dr. McCollum's laboratory. They consist of a wooden frame with galvanized iron-wire sides.

3. Wooden cages, made entirely of wood and glass.

Food Cups. Zinc cups were employed in the first two types of cages and glass cups in the third.

Drinking Water. The stock rats were given tap-water, the remaining rats were given doubly distilled water. This was first distilled through a copper still and then redistilled through glass. Animals receiving such water were given one drop per week per animal of the following solution:

Iodine	2 grams
KI	5 grams
H ₂ O	500 c.c.

The normal blood of the rat. Klineberger and Carl¹² give the following average counts for the blood of the rat (blood from thigh vein):

R. B. C. 9,300,000	P. M. N. 16.
Hb. 105% (Sahli)	P. M. E. 3.5
W. B. C. 15,200	L. lymph 24.5
	S. lymph 53.5
	L. M. 0.5
	T. 2.

100.0

These authors found no difference in the blood of the two sexes. The red blood cells of normal animals showed some anisocytosis and polychromasia. The leucocytes were predominantly small lymphocytes. The platelets tended to group themselves in masses.

Donaldson¹⁴ gives the following average counts for albino rats on a "scrap" diet.

R. B. C. 7,600,000—9,200,000	P. M. N. 44 to 71%
Hb. 85% to 100%	P. M. E. 0 to 3%
W. B. C. 7,200 to 16,000	P. M. B. 0
	Lymph. 30 to 55

Neither the source of the blood nor the type of the hæmoglobinometer used is stated.

Geiling and Green,¹⁵ in a preliminary report of some studies on blood regeneration in the rat on various diets, give the normal red blood count as between 7.5 and 10.5 millions and the hæmoglobin percentage as 110 to 140 (Smith-Cohen). They state that the number of blood cells per c.mm. in rats of different ages and sexes falls within these limits.

In discussing the blood of normal rats several factors should be taken into consideration: first, the species of rat used; second, the diet given as the stock diet; third, the presence of respiratory infections which are common in laboratory rats and which may modify the total leucocyte count and differential formula; and fourth, the source of the blood. White blood cell counts made on blood from the end of the tail are higher than counts made from venous blood and show a higher polymorphonuclear percentage. I also found that blood from the heart contained a uniformly lower white blood cell count than blood from the veins. The number of red blood cells and the percentage of hæmoglobin, however, were the same in blood from both these sources. It was necessary in some of the small rats to draw the blood from the heart, as a free flow could not be obtained from the cut vein. It should be remembered that the white blood cell counts are lower in these cases than if they had been made on blood from the thigh vein.

In our series of experiments the rats used were hybrids of the albino and the black Norway rat.

The diet employed as a stock diet for our animals was as follows:

Whole ground wheat
Cracked corn
Ground rolled oats 3 parts
Flaxseed meal 1 part

This was given *ad libitum* and in addition the rats received a small quantity of pasteurized milk and tap-water daily.

CHART I.

BLOOD COUNTS OF NORMAL RATS AT DIFFERENT AGES.

AGE	Blood from	Hb. %	R. B. C.	W. B. C.	DIFFERENTIAL						REMARKS
					Reticulated R. B. C. %	P. M. N.	P. M. E.	Lymph.	LM. T.	U.	
Newlyborn*	Heart	75	3,200,000	4,000	93	24	2	30	8	8	Mycocytes 4. Nucl. R.B.C. 250 in count- ing 100 W.B.C. } Anisocytosis. Polychromasia cor- responding to per- centage of Retic. R.B.C. 280 in count- ing 100 W.B.C. } R.B.C. See photo- graph.
Newlyborn				4,000		43	0	17	5	32	
15 Days	Heart	—	—	1,800	19	5	0	95	0	0	No Nucl. R. B. C. Marked leucopenia.
20 Days	Heart	—	—	3,600	20	2	0	90	8	0	No Nucl. R. B. C.
	Thigh vein	80	4,800,000	4,800	—	22	0	76	2	0	Rarity of P. M. N. forms.
26 Days	Thigh vein	85	5,120,000	3,600	18	12	0	76	4	8	
1 Mo.	Thigh vein	80	7,000,000	4,200	—	16	0	76	8	0	
	Thigh vein	85	6,592,000	9,000	6	24	0	74	2	—	
2 Mos.	Thigh vein	90	7,680,000	8,000	9	16	0	84	0	0	
4 Mos.	Thigh vein	110	11,200,000	9,600	3	24	6	68	2	0	
9 Mos.	Thigh vein	115	10,800,000	8,000	1	26	4	68	2	0	
10 Mos.	Thigh vein	110	9,856,000	12,000	—1	14	0	85	1	0	

* Under newly born are included rats under 24 hours old.

Chart I shows the results obtained in rats of different ages. The blood of a young rat differs from that of the adult, in that in the former the hæmoglobin content and the red blood cell counts are relatively lower, the white blood cell count is lower (a relative leucopenia) and the percentage of lymphocytes is relatively higher. Therefore, it is important in studying the blood of a rat of known age to compare it with a normal for its age. The normal count for a young rat would indicate a slight anæmia for an adult rat. We may, therefore, speak of a physiological anæmia in the nursing rat.

The diets which were used in these experiments may be roughly divided into two groups. The first of these included simple diets which had milk in one form or another as a basis. The second group includes the more complex mixtures of various purified foodstuffs. Rats were fed on

1. Pasteurized milk.
2. Pasteurized milk and bread.
3. Evaporated milk (Pet Brand).
4. Condensed milk (Eagle Brand).
5. Dried skimmed milk (Krystallak).
6. Dried skimmed milk with various additions.

A diet of pasteurized milk alone will not cause pathological changes in the blood of rats, and rats may be reared to the third generation on diets of milk and bread without developing anæmia.

Neither evaporated milk (Pet Brand) nor condensed milk (Eagle Brand) induced anæmia in rats even when the intake was limited to 8 c.c. and 4 gm., respectively, per diem in order to restrict the growth of the animals.

The attempt to cause anæmia by diets of dried skimmed milk (Krystallak) was a failure. Nor was anæmia produced by diets which contained Krystallak with yeast, butter fat or bread, or any combination of these additions to the skimmed milk diet. When, however, casein was added to a ration of Krystallak and butter fat, an anæmia was produced in two animals.

A. A female rat was started on a diet of
Krystallak 95
Butter fat..... 5
100

at the age of 25 days, when she weighed 30 grams. She was on the diet five months, during which time she gained in weight to 130 grams. At this time casein was substituted for 15 per cent of Krystallak:

Krystallak 80
Casein 15
Butter fat..... 5
100

The animal was kept in a galvanized iron cage on this diet for four weeks and lost 30 grams in weight. The rat appeared pale. Blood was taken from a thigh vein:

R. B. C.1,920,000
Hb.20% Leucopenia too marked
W. B. C.1,600 for accurate differential.

Red cells showed slight anisocytosis and poikilocytosis and there were many nucleated red cells. Polychromasia was fairly well marked. Approximately 70 per cent of the white cells were lymphocytes.

Autopsy. The tissues were very pale. The spleen was normal in size. The bones appeared normal and cut with resistance. Lungs normal.

B. A male rat, which was in cage with the above rat, was put on the same diet at the age of 25 days, when weighing 30 grams. He was on the diet five months, during which time he gained in weight to 150 grams. Fifteen per cent of casein was added, and the rat was kept on this diet for 6

weeks, on which he lost 20 grams in weight and developed pallor. Blood from thigh vein:

R. B. C.1,472,000	P. M. N.51
Hb.15—20%	Metamyelocytes 5
W. B. C.5,600	P. M. E. 5
	Eosinophilemyelocytes. 1
	Lymph.27
	T.1
	Unclassified10

100*

67 nucleated red cells were seen in counting 100 W. B. C. There was slight anisocytosis and poikilocytosis. Polychromasia was well marked.

Autopsy. As for above rat.

Microscopic sections: Bone. The cartilage was not calcified. There was a narrow subchondral zone of irregular trabeculae with a great deal of osteoid tissue. The cortex of the shaft was thickened and spongy. The marrow was congested.

The following types of complex diets were also studied to determine if possible the relations of certain deficiencies and excesses to the production of anemia.

7. Purified diets (a) without iron, (b) with iron.
 8. Diets low in fat-soluble A.
 9. Diets low in calcium.
 10. Diets low in calcium but with an excessive amount of fat-soluble A.
 11. Diets low in water-soluble B.
 12. Diets low in phosphorus and fat-soluble A.
 13. Diets low in calcium and in fat-soluble A.
 14. Other diets which produced changes in the bones.
7. *No anemia resulted in the first generation and only slight anemia in the second generation from a purified complete ration without added iron.*

For this series of animals a purified diet was used (indicated below as purified diet without iron).

Salt mixture.

Washed casein ..25**	NaCl 0.173
Butter fat10	MgSO ₄ 0.266
Agar 2	K ₂ HPO ₄ 0.954
Wheat germ.....10	CaH ₂ (PO ₄) ₂ .. 0.540
Salt mixture..... 3.58	Ca Lactate... 1.300
Washed dextrin...qs. ad 100	NaH ₂ PO ₄ 0.347

3.580

* In the formulas of the differential counts.

- P. M. N. = polymorphonuclear neutrophile leucocytes.
 P. M. E. = Eosinophile.
 Lymph. = Lymphocytes (large and small).
 T. = Transitional monocytes.

** I take pleasure here in thanking Dr. McCollum for outlining and analyzing for me this and the other complex diets which were used.

† The diets of the mother rats were never changed during lactation, that is, if the mother of a litter was on a deficient diet, she continued to receive it while the young were being nursed. The second generation animals received the same food as their parents, unless otherwise stated. Animals of the second generation on a faulty diet may be said, therefore, to have received the deficient food since birth, although it has been shown that the breast milk of a mother on a deficient diet is a better food for her young than the diet she receives is for her.

For the control series the identical diet (indicated below as purified diet with iron) was used except that ferrous lactate 0.118 grams was added to the salt mixture, bringing the salt mixture to 3.7 instead of 3.58%.

Both of these groups of rats were kept in wooden cages with paper for bedding and with glass doors and glass food and drinking cups. They did not come in contact with iron.

7a. Purified diet without iron.

A. A mother rat was put on the purified diet without iron at the birth of her young and remained on it while she nursed them.† Blood was examined from thigh vein of a male rat of this litter which had been on the same diet for 2½ months. His weight at this time was 250 grams.

R. B. C.12,240,000	P. M. N. 18
Hb.115%	Lymph. 70
W. B. C.12,000	T. 2
Reticulated R. B. C. 1%	Unclass. 10 (smudge cells)

100

At the age of 2½ months a splenectomy was performed under ether anesthesia. The spleen was larger than normal and weighed 0.91 grams. The animal recovered promptly and was kept 2½ months longer on this diet; weight 320 grams (total of 5 months on a diet); blood from thigh vein:

R. B. C.9,920,000	P. M. N. 7
Hb.110%	P. M. E. 6
W. B. C.40,000	Lymph. 84
Reticulated R. B. C. 3%	To 3

100

This animal had a severe respiratory infection.

Microscopic examination of the spleen showed a simple hypertrophy.

Autopsy. Subcutaneous fat abundant. The tissues were of good color. There was no infection about the wound and no hypertrophy of the lymphatic tissue. The bones were hard and normal in appearance.

B. A male rat of the same litter as the preceding rat on the same diet five months, but no splenectomy performed; weight 280 grams, blood from thigh vein:

R. B. C.12,300,000	P. M. N. 11
Hb.1.15%	P. M. E. 6
W. B. C.12,800	Lymph. 78
Reticulated R. B. C. 3%	T. 3
	Unclassified 2

100

Autopsy. The spleen was larger than normal, weighing 0.85 grams. Autopsy findings as in preceding rat.

7b. Purified diet with iron.

A. Control rat for the above. The mother of this rat was put on a purified diet with iron at birth of the young. A male rat of her litter was on this diet for 2½ months. Weight 250 grams. Blood counts at this time from thigh vein gave the following result:

R. B. C.11,872,000	P. M. N. 14
Hb.115%	P. M. E. 2
W. B. C.15,000	Lymph. 76
Reticulated R. B. C. 1-2%	T. 4
	Unclassified. 4

100

A splenectomy was performed under anesthesia; the weight of the spleen was 0.52 grams. The animal made a good recovery and was kept on this diet for 2½ months longer, making a total of 5 months on a diet. Weight 310 grams. Blood from thigh vein:

R. B. C.	9,920,000	P. M. N.	29
Hb.	115%	P. M. E.	9
W. B. C.	12,400	Lymph.	60
		T.	2

100

Microscopic examination of the spleen showed it to be normal.

Autopsy. Entirely negative, wound perfectly healed.

B. A male rat of the same litter as A, on the same diet for five months. No splenectomy performed; weight 290 grams; blood from thigh vein was counted as follows:

R. B. C.	12,640,000	P. M. N.	10
Hb.	115%	P. M. E.	1
W. B. C.	14,400	Lymph.	84
		T.	5

100

Autopsy. The spleen weighed 0.62 grams. Examination entirely negative.

The blood of two other rats of this series was examined with practically the same results. The blood of rats of the first generation on a purified diet without iron was found to be practically identical with that of rats on the same diet with iron added. The spleen of rats on the former diet, however, was uniformly larger than the spleen of those on the latter diet. Splenectomy had no effect on the blood picture.

Slight anemia resulted in some of the animals in the second generation receiving the purified diet without iron.

A. A female rat, whose mother had been on the purified diet without iron since her birth, at the age of 19 days weighed 21 grams. The blood of the mother was normal after the weaning of the litter. She had been on the deficient diet during lactation. Blood from thigh vein:

R. B. C.	4,112,000	P. M. N.	32
Hb.	45%	Lymph.	64
W. B. C.	3,400	T.	4
Reticulated R. B. C.	15-20%		

100

No nucleated red cells were seen. There was slight anisocytosis, no poikilocytosis, but fairly marked polychromasia.

Autopsy. Spleen normal in size, weight 0.77 grams, tissues somewhat pale, bones cut with resistance.

B. A young rat, whose mother had been on a purified diet without iron since her birth, at the age of 42 days weighed 29 grams. Blood from thigh vein:

R. B. C.	6,400,000	P. M. N.	40
Hb.	60%	Lymph.	60
W. B. C.	4,600	T.	0
Retic. R. B. C.	15%		

100

• The red cells showed moderate polychromasia, otherwise they were normal.

Autopsy. The spleen was small, weighing 0.1 gram. Liver and tissues fairly good color. Lungs normal. The bones cut with resistance.

C. One of the young of a mother rat which had been on a purified diet without iron since birth, at the age of 24 days, weighed 25 grams. Blood from thigh vein:

R. B. C.	7,360,000	P. M. N.	28
Hb.	67%	Lymph.	72
W. B. C.	7,600	T.	0
Retic. R. B. C.	10%		

100

Slight aniso- and poikilocytosis and polychromasia. No nucleated R. B. C. seen. No autopsy.

D. A young rat of the same litter as the preceding but aged 2 months. Weight 110 grams. Blood from thigh vein:

R. B. C.	not done	P. M. N.	14
Hb.	100%	Lymph.	86
W. B. C.	5,600	T.	0
Retic. R. B. C.	8%		

100

Slight polychromasia, red cells otherwise normal in appearance.

Autopsy. Tissues and viscera of good color. The spleen was slightly enlarged, the thymus persistent and bones cut with resistance.

E. Mother rat was put on purified diet with iron at birth. Blood of mother normal after weaning litter. One female of this litter at the age of 19 days weighed 32 grams. Blood from thigh vein:

R. B. C.	6,144,000	P. M. N.	10
Hb.	80%	Lymph.	88
W. B. C.	3,400	T.	2
Retic. R. B. C.	10%		

100

There was a slight anisocytosis and polychromasia. No nucleated red cells were seen.

Autopsy. The spleen was normal in size, weighing 0.1 gram; the thymus somewhat enlarged. The bones were hard and cut with resistance.

F. Rat, aged 1 month, weight 40 grams. Third generation on purified diet with iron. Blood from thigh vein:

R. B. C.	10,240,000
Hb.	110%
W. B. C.	2,600

The red cells appeared normal and the differential blood picture was essentially normal.

No autopsy.

7a. Diet 2.

Casein	12.5
Butterfat	10.0
Wheat germ	5.0
Agar	2.0
Salt mixture without iron..	3.7
Dextrin	67.0

100.0

The diet was made of purified food stuffs. It was iron free and had about half the protein content of the last mentioned diet. Two generations of rats were reared on this diet. Four rats of the second generation, weighing 33 to 40 grams, were kept on this diet from the age of 30 days for three months. At the end of this period they weighed 130 to 150 grams. on purified diet with iron. Blood from thigh vein:

R. B. C.	10,880,000
Hb.	90%
W. B. C.	7,000

The smears showed red blood cells which were apparently normal. The differential count was normal.

Autopsy. Tissues and viscera of good color. The spleen was enlarged; the bones appeared to be normal.

Microscopic sections of the bones of rats raised on the above purified diets showed practically normal bones. The cartilage was well calcified but the trabeculae were somewhat fewer in number than normal, i. e., the bones were slightly osteoporotic.

8. *The blood of rats on a diet so deficient in fat soluble A as to produce xerophthalmia showed no anemia.*

The diet which was used had the following composition.

Rolled oats.....	40.0
Flaxseed oil meal.....	8.3
NaCl	1.0
CaCO ₃	1.5
Dextrin	49.2
<hr/>	
100.0	

9. *The following diets were adequate in all respects except for a deficiency in calcium.*

Wheat	20.0
Maize	15.0
Rice	9.5
Rolled oats	9.5
Peas	10.0
Navy beans	10.0
Casein	10.0
Whole milk powder	5.0
NaCl	1.0
Butter fat	10.0
<hr/>	
100.0	

Wheat	20.0
Maize	19.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
NaHCO ₃	1.5
Butter fat	10.0
<hr/>	
100.0	

The blood of rats fed on these diets was quite normal.

10. *This was also true of the blood of rats which were kept on the following diet which contained an excessive amount of calcium and fat.*

Wheat	20
Maize	10
Rice	9
Rolled oats	9
Peas	9
Navy beans	9
Casein	10
NaCl	1
CaCO ₃	3
Butter fat	20
<hr/>	
100	

11. *Rats on a diet so deficient in water soluble B as to produce polyneuritis were not anemic but showed a leucopenia with evidences of diminished leucopoietic activity.*

The diet had the following composition:

Casein	18
*Salt mixture No. 185.....	3.7
Agar	2.0
Butter fat	5.0
Dextrin	71.3

100.0

A. A male rat was put on this diet, at the age of 55 days, when weighing 70 grams. At the end of 137 days he weighed 65 grams. In a state of polyneuritis. Blood from thigh vein:

R. B. C.	10,320,000	P. M. N.	19
Hb.	100%	Lymph.	35
W. B. C.	2,600	T.	1

55

The red cells appeared normal, the polymorphonuclear cells were chiefly cells with 5 or 6 lobed nuclei. The nuclei often filled the entire cells.

B. A male rat, weighing 56 grams, was put on diet at the age of 50 days. At end of 114 days weighed 50 grams. Animal in a state of polyneuritis. Blood from thigh vein:

R. B. C.	10,000,000
Hb.	110%
W. B. C.	1,800

The smears showed very marked leucopenia; the red cells appeared normal. The white cells were about equally divided between polymorphonuclears and lymphocytes. The polymorphonuclear cells had multilobulated nuclei.

Autopsies. These rats with polyneuritis were poorly nourished rats. The spleens were of normal size. Thymus glands atrophic. Tissues of good color and the bones hard on section.

Microscopic section of the bones of these animals showed that the cartilage was thin and well calcified. Few trabeculae were visible. The marrow was hemorrhagic.**

Findlay¹⁶ described the same changes in the blood in beriberi in man as we have found in rats, namely, leucopenia and a shift to the right in the Arneth formula without important changes in the red cells and hemoglobin.

12. *Diets which are relatively high in calcium but which are low in phosphorus and in an uncharacterized substance which is present in certain fats do not produce anemia in rats.*

McCollum, Simmonds, Shipley and Park have described a very severe form of rickets which is produced by diets which have the above described characteristics. The animals whose blood was studied were fed on their diet No. 3143 which was made up as follows: †

Wheat	33.0
Maize	33.0

* Salt mixture No. 185 is the same as the salt mixture above in the purified diet without iron.

** The pathological condition of the bone and bone marrow of these rats with polyneuritis is described by Shipley, McCollum and Simmonds, Jour. Biol. Chem., Dec., 1921.

† The blood counts on these animals were made by Dr. A. A. Weech.

Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
CaCO ₃	3.0

The blood of four animals on this diet was studied. The diet did not produce anaemia in any case and the leucocytes were quite normal.

13. *Diets low in calcium, normal or relatively high in phosphorus and low in a substance contained in certain fats caused bone changes in rats which were closely related to rickets. These diets also produced anaemia in both first and second generations.*

A brief review of the work of Shipley, Park, McCollum and Simmonds may serve to make the following discussion clearer. These authors have found that on diets in which an organic substance, present in cod-liver oil and to a less extent in butter fat, which may or may not be identical with fat soluble A, is small in amount and in which there is a disproportion between the calcium and phosphorus, a condition of the bones results which is practically identical with human rickets. Park and Shipley have frequently noted in these animals varying degrees of enlargement of the spleen and marked pallor of the viscera particularly of the liver. The rats whose blood counts are recorded below were from their series and had been in cages made of wood and galvanized iron. The blood of rats on six diets differing in composition but with the same faults were examined.

Six rats, aged 45 days, whose average weight was 45 grams, were put on the following diet which contains a poor fat and an insufficient quantity of calcium but an abundance of phosphorus:

Wheat	30.0
Maize	19.5
Peas	8.5
Rolled oats	8.5
Rice	9.5
Navy beans	8.5
Casein	10.0
NaCl	1.0
NaHCO ₃	1.5
Cottonseed oil	3.0

100.0

A. One female was examined at the beginning of the experiment. Blood from her thigh vein was examined with the following result:

R. B. C.	7,928,000	P. M. N.	9
Hb.	92%	Lymph.	82
W. B. C.	4,700	T.	9

100

Slight polychromasia, no anisocytosis or poikilocytosis.

B. A male was examined after receiving the diet for 3 months. Blood from his thigh vein showed:

R. B. C.	10,208,000	P. M. N.	22
Hb.	100%	P. M. E.	2
W. B. C.	14,400	Lymph.	74
Retic. R. B. C.	8%	T.	2

100

Slight polychromasia and anisocytosis were present.

Autopsy. The spleen was of normal size. The tissues were of good color. The thorax was much deformed.

Microscopic sections. There was a great overproduction of osteoid tissue in the epiphysis and the shaft was irregularly calcified and was invaded by blood vessels. There were large numbers of trabeculae in the shaft each surrounded by a broad zone of osteoid. The cortex of the shaft was thickened. The spaces between the trabeculae near the marrow cavity contained large numbers of basophilic cells. The spleen showed marked congestion. The Malpighian bodies were larger than normal.

C. A female was examined after being on the diet for 4½ months. She weighed 84 grams. Blood from thigh vein showed

R. B. C.	9,600,000	P. M. N.	10
Hb.	110%	Lymph.	88
W. B. C.	9,000	T.	2
Reticulated R. B. C.	less than 10%.		100

The red cells showed slight polychromasia, otherwise they were normal.

Autopsy. The spleen was slightly enlarged. The thorax was deformed and the costo-chondral junctions were enlarged. The bones cut easily and showed broad metaphyses.

Microscopic section. The changes in the bones were similar to those just described. The Malpighian bodies of the spleen were enlarged and contained nests of erythropoietic cells.

D. A female was examined after being on the diet for 6 months. The animal was pale and weighed 80 grams. Blood from thigh vein:

R. B. C.	2,608,000	P. M. N.	37
Hb.	25%	Lymph.	61
W. B. C.	12,000	T.	2
Retic. R. B. C.	50%		100

Eighteen nucleated red cells were seen in counting 100 white cells. There was a marked polychromasia. The polychromatic red cells corresponded with the number of reticulated red cells (see Figs. II, III, IV). Anisocytosis and poikilocytosis were fairly well marked.

Autopsy. The spleen was large, weighing 0.8 grams. The liver and other tissues were very pale. There was marked rickets. The bones cut easily and showed broad metaphyses.

Microscopic section. The bone showed more extensive rickets than in the preceding animal. There was no calcification of cartilage, which was very irregular (see Figs. V and VI).

The Malpighian bodies of the spleen were fewer in number than in the spleen of the preceding rat. There was infiltration with round cells and large mononuclear cells. There was myeloid metaplasia of the spleen with evidences of the assumption of hematopoietic function.

Summary. A rat fed upon a defective diet for 6 months had severe rickets, splenomegaly, and actively regenerating secondary anemia.

E. A rat of another litter fed upon the above defective diet from birth at age of 2 months weighed 30 grams. Blood from the thigh vein:

R. B. C.	7,360,000
Hb.	110%
W. B. C.	2,200
Reticulated R. B. C.	10%

The red cells showed slight polychromasia. There was leucopenia with a normal differential formula.

Autopsy. The spleen was normal. There were signs of early rickets.

F. A female rat, at the age of 40 days, when weighing 55 grams, was put on the following diet which is deficient in calcium and fat:

Wheat	25.0
Maize	19.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
Dextrin	1.5
Whole milk powder	5.0

100.0

At the end of 7½ months her weight was 83 grams. Blood from the thigh vein contained:

R. B. C.	5,232,000
Hb.	60%
W. B. C.	10,000
Reticulated R. B. C.	24%

The red cells showed moderate polychromasia, marked anisocytosis and some poikilocytosis. A few nucleated red cells were seen. About 75 per cent of the white cells were lymphocytes. There were no myelocytes in the blood film.

Autopsy. The thorax was much deformed. The spleen was slightly enlarged, weighing 0.55 gram.

Microscopic section. The bones showed rickets in process of healing. The spleen showed evidences of hematopoiesis.

Summary. A moderate anæmia for a rat of this age with active regeneration of the red cells. Slight enlargement of the spleen. Rickets.

A. A female rat, aged 45 days and weighing 65 grams, was fed upon the following diet, which is also deficient in calcium and fat:

Soy bean	30
Wheat gluten	5
Casein	10
Wheat germ	5
NaCl	1
Maize	20
Dextrin	29

100

Her weight was 75 grams at the end of 78 days. Blood from thigh vein:

R. B. C.	3,120,000	P. M. N.	22 (incl. 2 meta- myelocytes)
Hb.	35%		
W. B. C.	13,000	P. M. E.	0
Retic. R. B. C.	75-80%	Lymph.	78

100

Seventy-five nucleated red cells were seen in counting 100 W. B. C. The red cells showed anisocytosis and poikilocytosis and marked polychromasia (see Figs. VII and VIII).

Autopsy. The spleen was very much enlarged. There were marked changes in the skeleton. The cartilage was redundant. Calcification was very defective and irregular. The metaphysis consisted of osteoid tissue in the form of trabeculae, somewhat irregularly arranged. At the periphery of the bone, processes of unchanged cartilage ran down from the cartilage into the metaphysis. There were small islets of cartilage cells in the metaphysis. The cortex of the shaft was spongy. There was a great deal of osteoid tissue around all of the trabeculae in the spongiosa. There were no signs of abnormal resorption in the metaphysis but resorption was going on actively in

the cortex and in the spongiosa near the marrow cavity (see Figs. IX and X).

Summary. Rachitic rat; enlarged spleen; severe secondary anæmia; evidences of active regeneration of red cells.

13. *Anæmia was caused in the second generation of rats fed upon the following diets that produced changes in the bones.*

A. A male rat, aged 41 days, weighed 22 grams. The mother was on the following diet for 217 days before birth of her litter:

Wheat	19.3	
Maize	20.0	
Rice	10.0	This diet is deficient in
Rolled oats	10.0	calcium, while contain-
Peas	10.0	ing a fat which is not
Navy beans	10.0	strongly protective
Casein	10.0	against rickets. There
NaCl	1.0	is a relatively large
NaHCO ₃	1.5	amount of phosphorus.
CaCO ₃	0.2	
Butter fat	8.0	

100.0

Blood from heart:

R. B. C.	448,000
Hb.	less than 10%
W. B. C.	1,000
Reticulated R. B. C.	70%

The red cells showed marked anisocytosis and polychromasia and some poikilocytosis. A few nucleated red cells were seen. There was leucopenia with many early forms of the bone marrow series.

Autopsy. The tissues were pale. The liver was nearly white. The spleen was pale, but not enlarged. There were no gross signs of rickets.

B. A female rat, aged 24 days, weighed 18 grams. The mother had been on the following diet 60 days before the birth of her litter:

Rice	10.0	
Wheat	32.5	
Maize	15.0	
Peas	10.0	This diet is deficient
Navy beans	10.0	in calcium and fat.
Rolled oats	10.0	
Casein	10.0	
NaCl	1.0	
NaHCO ₃	1.5	

100.0

Blood was taken from the heart. The blood was thin and watery, and enough to count could not be obtained. Smears were made which showed fairly well marked anisocytosis and poikilocytosis and polychromasia. Many nucleated red cells were seen. There was a leucopenia.

Autopsy. The tissues and viscera were very pale. The spleen was not enlarged.

C. A female rat, aged 16 days, weighed 10 grams. The mother had been on the above diet for 84 days before the birth of her litter. Blood from heart:

R. B. C.	5,044,000
Hb.	75%
W. B. C.	1,200
Reticulated R. B. C.	10%

The red cells showed slight polychromasia. There was a marked leucopenia, but the white cells seen appeared to be normal. No nucleated red cells were seen.

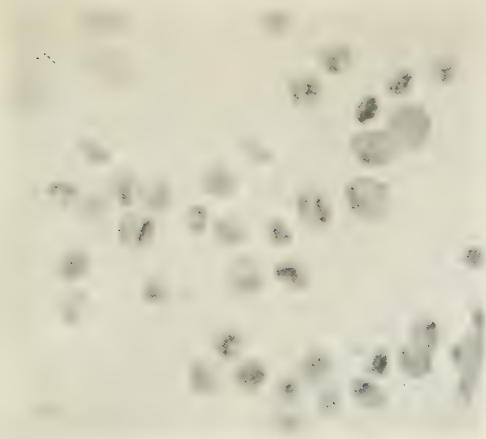


Fig. I.

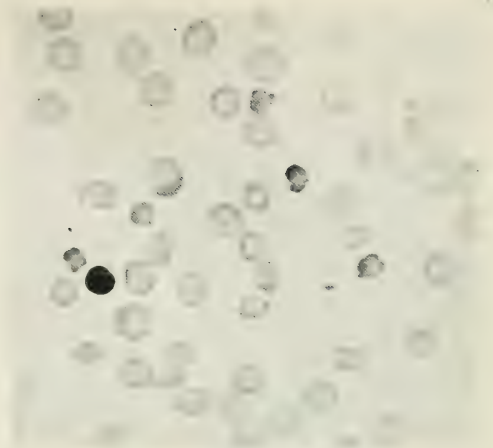


Fig. II.



Fig. III.

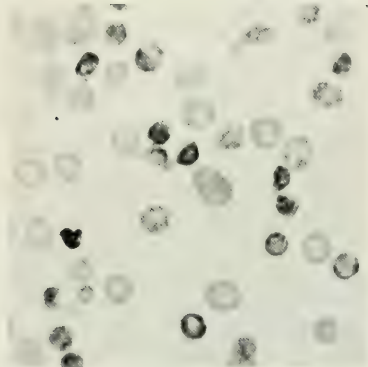


Fig. IV.

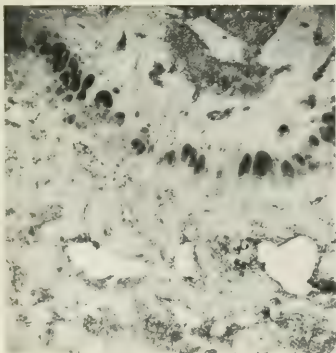


Fig. V.

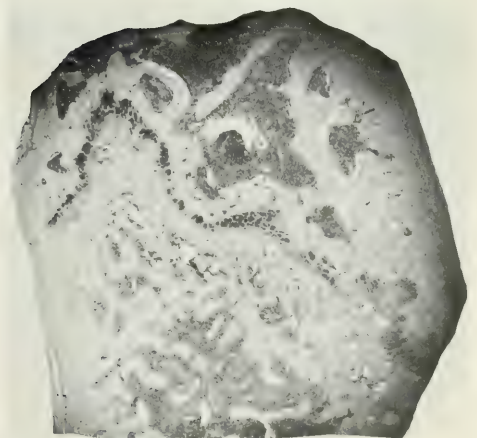


Fig. VI.

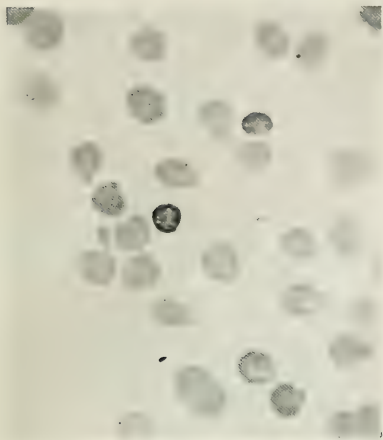


Fig. VII.

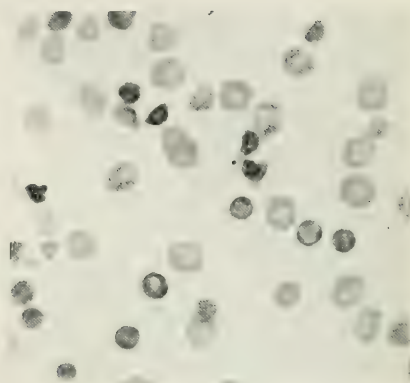


Fig. VIII.

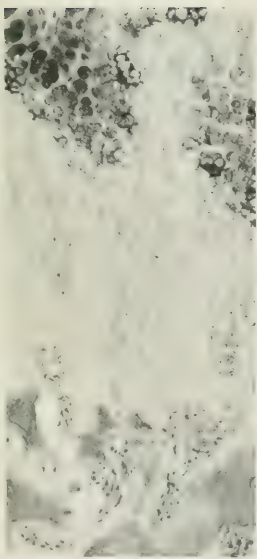


Fig. IX.



Fig. X.

Autopsy. The spleen was small. There was no thoracic deformity. The costo-chondral junctions were slightly enlarged. The long bones were thin and cut easily.

Microscopic sections showed well calcified osteoporotic bones.

D. A female rat, aged 19 days, weighed 14 grams. The mother of this animal had been on the following diet 70 days. She continued on the diet while she nursed her litter:

Wheat	30	
Maize	30	
Rice	10	This diet is deficient
Rolled oats	10	in calcium and fat.
Peas	10	
Navy beans	10	

100

Blood from thigh vein:

R. B. C.	6,208,000	P. M. N.	15
Hb.	70%	Lymph.	80
W. B. C.	not counted	T.	5

100

There was anisocytosis, poikilocytosis and a moderate degree of polychromasia. An occasional nucleated red cell was seen. The animal had a marked leucopenia.

The bones of the mother showed typical rickets. The bones of the second generation rat showed no osteoporosis and no rickets.

In five other rats of the second generation a practically normal blood picture was observed.

DISCUSSION

A diet deficient in iron alone does not produce anæmia in the rat in the first generation. It would appear either that the rat is born with a store of iron sufficient to carry him through life, or that only a very small quantity of iron is necessary to meet his requirements. On the other hand, diets deficient in an organic substance contained in cod-liver oil and to a less extent in butter fat and which also have certain improper calcium-phosphorus ratios (diets which produce rickets in the rat) produce anæmia in some animals. The anæmia in these animals is not due simply to a deficiency of iron in the diet. While these diets contained only a small amount of iron, they contained as much of this element as the stock diet or the other diets used upon which no anæmia developed. It would seem that the disturbance in the inorganic metabolism resulting from certain diets with which there is a lack of calcification of the bones also resulted in, or was accompanied by, a disturbance of the iron metabolism. It will be noted that the phosphorus content was relatively normal or increased and calcium relatively low in all the diets upon which anæmia developed.

When, however, the diet contained relatively excessive amounts of calcium but was low in phosphorus, no anæmia was produced in the rats which I examined. Diets of this type cause an exaggerated form of rickets to develop in rats and the calcium and phosphate content of their blood is identical with that seen in many severely rachitic children.

Milk diets except those in which casein was present in high percentage did not produce anæmia. Two rats which received the latter diet were the only animals in

the series on diets of milk or modified milk which showed profound disturbance of calcification of the bones. I have no explanation to offer for this at present.

Enlargement of the spleen appeared to be as variable a feature in rats with rickets as it is in children with rickets. Enlargement of the spleen was frequently observed in rats in association with an anæmia of the actively regenerating type just as splenomegaly is commonly observed in infants with rickets and anæmia. However, although rickets makes its appearance relatively early in the rat, especially when the animal is put on the faulty diet at an early age, anæmia does not make its appearance until the rat has been on the diet for a long period. For this reason it is difficult to study anæmia produced by faulty food in rats, since they usually die from malnutrition or embarrassed pulmonary or cardiac function resulting from the marked thoracic deformity, or from pulmonary infection, before there is time for the anæmia to become manifest. The rats in which anæmia occurred had been on the faulty diet for a long period, or the anæmia was observed only in the second generation. Rachitic rats are usually sterile, so that second generations are not often raised.

The examination of films of the blood of the same rat, made simultaneously, the one stained with cresyl blue and Wilson's stain following the technique outlined above, the other with Wilson's stain alone, showed that if the polychromatophilic (diffusely basophilic) red blood cells and the reticulated erythrocytes were counted, the counts were practically the same. This would seem to indicate, at least as far as the rat is concerned, that polychromasia of the red blood cells is an indication of a young cell. Because of the stimulation of the bone marrow which occurs in the course of anæmia of the type described in these rats, these immature cells are poured into the circulation in large numbers. They compose a large percentage of the red blood cells present. In the blood of the normal adult rat they are seldom encountered.

Although the anæmic animals were not treated, it would seem logical to assume that these anæmias require anti-rachitic therapy primarily. Iron would not seem to be primarily indicated because the amount of iron in any well balanced diet is sufficient to meet an animal's needs.

CONCLUSIONS

1. The normal blood picture of the rat varies with age. Young rats have a relatively lower red blood cell count, white blood cell count, and hæmoglobin percentage, and a higher lymphocytic percentage, than the adult rat. Diet and the presence of respiratory infections are factors which may influence the blood picture.

2. Well balanced diets, deficient in iron, do not produce anæmia in the rat in the first generation, nor do diets consisting solely of cow's milk or milk and bread. Slight anæmia may occur in rats of the second generation on these diets.

3. Diets deficient in fat soluble *A* or water soluble *B*, although they cause severe nutritional disturbances, do not produce anaemia in the rat. Diets so deficient in water soluble *B* as to produce polyneuritis diminish leucopoietic activity and cause a severe leucopenia with a shift to the right in the Arneth formula.

4. Diets low in an organic substance contained especially in cod-liver oil with a low calcium but high phosphorus content, which produce rickets-like changes in the rat, may also produce anaemia, provided the animal is kept for a long period on the diet. Animals of the second generation on this diet may also become anaemic. This anaemia is associated with evidences of increased hematopoietic activity. There is often an enlargement of the spleen. This condition resembles the anaemias seen in association with rickets in human beings.

5. A diet low in the organic substance contained in cod-liver oil and low in phosphorus with a normal calcium content, a diet that produces severe rickets with great uniformity, does not produce anaemia.

LEGENDS FOR FIGURES.

Fig. I.—Blood of normal newly born rat, stained with cresyl blue and Wilson's modification of Romanowsky's stain, showing the high percentage of reticulated red blood cells present.

Figs. II and III.—Blood of rat D, (p. 171), stained with Wilson's modification of Romanowsky's stain, showing one nucleated red cell and polychromasia of many red cells.

Fig. IV.—Blood of same rat stained with cresyl blue and Wilson's modification of Romanowsky's stain, showing reticulation of the red cells.

Figs. V and VI.—Sections of femur of same rat at different magnifications, showing rickets.

Fig. VII.—Blood of rat A (p. 170), stained with Wilson's modification of Romanowsky's stain, showing polychromasia.

Fig. VIII.—Blood of same rat stained with cresyl blue and Wilson's modification of Romanowsky's stain, showing reticulation of the red cells.

Figs. IX and X.—Sections of femur of same rat at different magnifications, showing rickets.

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THE SIGNIFICANCE OF THE INFLUENZA BACILLI

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Two main problems exist in regard to the influenza bacillus:—(1) Is it the cause of epidemic influenza, and (2) if not, what relation does it have to the disease and what is its general significance? The first of these questions, as will be pointed out below, we regard as settled—in the negative. It is with the second that this communication mainly concerns itself.

Is the influenza bacillus the cause of epidemic influ-

enza? During the great pandemic of 1889-1891 Pfeiffer¹ noticed in smears from the pharynx and sputum of patients ill with the disease the organism which now bears his name. Briefly stated, the facts brought out by Pfeiffer and his associates were as follows: The bacillus could be found with great regularity in the secretions from the respiratory tract in cases of influenza, it was a small, gram-negative, aerobic organism requiring hæmoglobin quished true and pseudo-influenza bacilli on the basis of

for its growth on artificial media. Pfeiffer at first distinguished morphology, the latter being larger and tending to grow in long threads, but later he seems to have concluded that all "influenza bacilli" were identical. One wonders now if Pfeiffer's pseudo-influenza bacillus was not the organism at present known as bacillus X or hemolytic influenza bacillus². Studied as it was towards the end of the pandemic without there being ample opportunity for confirmatory work, nevertheless the organism of Pfeiffer seems at the time to have been generally regarded as the cause of the disease. In the light of present knowledge about this group of bacilli, it seems certain that Pfeiffer was dealing with no specific organisms, nor have we more than the bare fact of their association with influenza cases to support the idea of their etiological rôle in the previous pandemic.

During the twenty-five years which elapsed between the last two great epidemics one came to think of the influenza bacillus in a rather different way. It became apparent that it was not constantly present in the inter-epidemic outbreaks of mild influenza-like disease or grippé. Davis,³ for example, in the epidemic of 1908 found influenza bacilli in four of twenty-three cases, and Lord⁴ in three of twenty cases, while Moody and Capps⁵ (1916),—in a careful study of thirty-one severe instances of grippé, found them only twice. On the other hand it appeared that influenza bacilli could be isolated from normal people,^{6, 7} and from a variety of pathological processes. Scheller⁸ summarizes the literature on the occurrence of these organisms as follows:—epidemic influenza, the entire respiratory tract from nose to alveoli, tonsils-inflamed or normal, sinuses, middle ear infections, pleurisy, arthritis, pyelitis, orchitis, epididymitis, cystitis and urethritis; pyo- and hydro-salpinx, cholecystitis, tuberculosis (secondary), bronchiectasis, meningitis, and as part of a mixed infection in diphtheria, measles, scarlet-fever and whooping-cough (?). Needless to say all of these observations were made on a basis of morphology, staining, and simple cultural characteristics without attempt at adequate biological classification. During a rather extensive experience with clinical respiratory bacteriology from 1912 to 1918, we encountered influenza bacilli frequently in all types of acute and chronic respiratory infection. One is especially impressed by the tendency of the group to associate themselves with other bacteria. For example, we have on several occasions in cases of frank lobar pneumonia isolated *B. influenza* in pure culture from sputum when the lung puncture yielded pneumococcus alone. Luetscher's⁹ experience in the course of careful observations on a large series of respiratory infections was a similar one. In 603 cases he isolated influenza bacilli in 28.5%. Lord¹⁰ reports findings of the same kind. Before the recent pandemic, therefore, one would have been inclined to regard the influenza bacillus as a rather ubiquitous organism in the diseased

and normal respiratory tract, unfolding its activities especially on tissues damaged by other infections. One may add that at this time no observations of consequence in regard to the biological relationship of the group were on record save perhaps those of Wollstein¹¹ who found some difference between meningitic strains and those from the respiratory tract.

The appearance of the pandemic in 1918 immediately revived an intense and general interest in the influenza bacillus. We shall discuss the voluminous literature of the past three years only from the point of view of its bearing on the etiology of the disease. Three main arguments have been brought out by those who believe the influenza bacillus to be the cause of epidemic influenza,—(1) The frequent presence of the organism in cases of the disease; (2) The production of experimental influenza in animals and in man by inoculation with the bacillus; and (3) The development of immunity reactions.

That the influenza bacillus was present during the recent pandemic with great constancy both in uncomplicated cases of influenza and in those followed by pneumonia is established beyond dispute. The uniformity of this finding followed directly the introduction of suitable media for the cultivation of the organism, especially the chocolate medium of Williams, and the oleate hæmoglobin agar of Avery. One need only quote such reports as those of Williams,¹² of Keegan,¹³ of Rappaport,¹⁴ and of Stillman and Pritchett¹⁵ who found influenza bacilli in from eighty to one-hundred per cent of the cases as illustrations of the usual experience. We have found no records of work done during the epidemic with proper media and facilities which do not agree with this result. It does not seem fair, however, to draw definite etiological conclusions from such observations. By an exactly similar line of reasoning the micrococcus catarrhalis or the streptococcus viridans might be said to be the cause of influenza. One must recall in this connection the well known example of the hog-cholera bacillus.

The transmission experiments must be closely scrutinized. Blake and Cecil¹⁶ report the production in monkeys, by inoculation with virulent influenza bacilli of a disease which they regard as identical with human influenza. While they have undoubtedly produced a pulmonary infection with the organism, we are not at all impressed by its similarity to *uncomplicated* human influenza. This experimental disease is perhaps roughly comparable to the *post-influenzal* pneumonias in man. The same scepticism may be entertained in regard to the experiences of Cecil,¹⁷ and of Park,¹⁸ who inoculated human beings. Mild indispositions with catarrhal symptoms, such as followed some of the inoculations, are too dubious to be regarded definitely as true influenza.

The immunity reactions which have been reported by some observers indicate no more than a reaction to the influenza bacillus.^{19, 20, 21, 22, 23, 24, 14.} They have no definite bearing on its causal rôle in epidemic influenza.

In summary, then, recent studies have failed to bring satisfactory proof that the influenza bacillus is the cause of epidemic influenza.

On the other hand, certain observations seem to weigh strongly against the case for the Pfeiffer bacillus. As early as the epidemic in 1918 Bloomfield and Harrop,²⁵ on clinical grounds, felt that the influenza bacillus could not be the cause of the disease. It seemed to us inconceivable that an organism which was so ubiquitous and so versatile in its activities and, as a rule, so obviously a secondary invader, could suddenly produce a disease of fixed and definite characteristics and one so essentially different from the known influenzal lesions. This view, needless to say, was held in regard to the primary disease and not the secondary pneumonias. Furthermore, the incidence of the Pfeiffer bacillus was found to be practically as high in other acute diseases occurring at the time of the epidemic as in influenza itself. One may mention, for example the observations of Sellards and Sturm,²⁶ who found influenza bacilli in twenty-five of thirty-one cases of measles. Again, transmission experiments conducted under such ideal conditions as those of Rosenau²⁷ and of Leake²⁸ cannot be altogether disregarded, although it must be admitted that negative results are never conclusive. But most important are the serological studies of Park and his associates^{29, 30} of Coca,³¹ of Bell³² and of Chesney.³³ These men all failed to find that various strains of influenza bacilli from epidemic cases were identical, when tested by biological methods such as agglutination. As first emphasized by Park,³⁴ one can hardly believe that the pandemic could have been caused by anything but a highly specialized strain of the causal organism. Finally, the recent studies of Gates and Olitzky,³⁵ although as yet unconfirmed by others, are most suggestive. The finding of a filter-passing organism in the secretions of epidemic cases, which reproduces rather constant lesions in animals, deserves most careful consideration.

On the whole, then, it seems to us that the influenza bacillus or rather the group of so-called influenza bacilli can no longer be regarded as the cause of the epidemic disease.

What then is the nature of the relationship of the influenza bacillus to epidemic influenza, and what in general is its significance? It is important at this point to emphasize the fact now clearly established that "influenza bacilli" really represent a great group of organisms and not a single fixed type. The literature on this question is adequately reviewed by Rivers,³⁶ and by Stillman and Bourn.³⁷ In brief it may be said that while the organisms in question have certain common characteristics—to wit, that they are all small Gram-negative bacilli, hemoglobinophilic on the ordinary laboratory media,³⁸ when classified by biological methods such as agglutination as well as by simpler criteria such as the

production of hemolysis, indol formation, reduction of nitrates, amylase formation, and sugar fermentations, they fall into a large variety of sub-groups, the extent of which is as yet unknown.

None the less it is certain that as a whole influenza bacilli were tremendously prevalent at the time of and following the epidemic of 1918 both in patients with influenza and in other individuals. A comparison of the incidence before and after the epidemic can unfortunately not be made, inasmuch as the inadequate cultural methods previously used would hardly allow a fair estimate. It seems to us, however, that light may be shed on the question of the significance of the group by studying the occurrence of Pfeiffer bacilli now—three years after the pandemic—at a time when influenza once more seems extinct.

Briefly stated, then, the idea of the present study was as follows:— Even if one makes allowance for the inadequacy of bacteriological methods before the epidemic of 1918, it seems certain that at that time there was no widespread existence of influenza bacilli in normal healthy people. Davis,⁶ in 1907, found them in two of twenty normal throats, and Holt⁷ (1910) reports their presence in nineteen per cent of two hundred and fifty-four persons without respiratory disease. On the other hand, their incidence in pulmonary lesions as secondary invaders was high.^{9, 10} But at the time of the epidemic their prevalence became wide, not only in cases of influenza but in normal individuals as well. Lord³⁹ in the midst of the pandemic found influenza bacilli in seventy-six per cent of thirty-four normal students, Pritchett and Stillman¹⁵ in forty-two per cent of controls, Opie⁴⁰ in thirty-five per cent, and Jordan⁴¹ in forty per cent. Even during the first two years after the epidemic the incidence in normals remained high—Winchell and Stillman⁴² (39 per cent), Pilot and Pearlman⁴³ (40 per cent) and Bloomfield⁴⁴ (90 per cent). It seemed of interest, therefore, to determine the occurrence of Pfeiffer bacilli now (1921-22) three years after the epidemic. Should it turn out that the incidence had dropped to a figure comparable to the pre-epidemic results, one would be forced to conclude that the presence of influenza alters the respiratory mucous membrane as a soil in such a way that *B. influenzae* becomes temporarily adapted to a widespread saprophytic growth. Such a fact would be of interest not only on its own account but because of its general bearings on questions of bacterial adaptation.

In summary we have studied the following questions:—

1. The comparative incidence in the population at large during epidemic, post-epidemic, and later periods.
2. The seasonal variation, if any, through the year in relation to respiratory disease.
3. The relative number of chronic and of temporary carriers.
4. The localization of organisms in carriers in relation to persistence.

of colonies so that accurate counts can be made. Each batch of medium was tested before use by inoculation with stock strains of influenza bacilli. Unless luxuriant growth was obtained the medium was discarded. We regard this test as very important, since successive batches of Avery medium, even if prepared in the same way, may not be equally satisfactory. Suspicious colonies were identified by staining and by subculture on plain agar and on rabbit blood-agar.

Before Pandemic of 1918	During Pandemic	During two years after Pandemic	Present Incidence
Davis 10% Holt 19%	Lord 76% Pritchett & Stillman 42% Opie 35% Jordan 40%	Winchell & Stillman 39% Pilot & Pearlman 40% Bloomfield 90%	Total cultures 283 Number positive 49
Average— not over 20%	Average — about 50%	Average — about 50%	Percentage positive 17.3%

[illegible][illegible]

RESULTS

1. *The Comparative Incidence of Influenza Bacilli at Various Times.* It was our aim in this part of the study to make single cultures in a large group of healthy individuals free from respiratory disease in order to determine the incidence of the influenza bacillus now, in comparison with its occurrence before, during, and after the pandemic. During the months of August to December 1921 a miscellaneous group of two hundred and eighty-three people was examined. Of these, forty-nine, or 17.3%, yielded Pfeiffer bacilli in numbers varying from a few colonies up to almost pure plates. Table I shows figures derived from the literature in comparison with our present results. It is seen that the increase which occurred at the time of the epidemic persisted for about two years, but that now (1921-22) the incidence is much lower,—in fact quite comparable to that which existed prior to the pandemic. This point can be brought out still more clearly (Table II) by comparing in detail two sets of cultures of our own, the first made during the spring of 1920, the second during the winter of 1921-22. Exactly the same methods were used and the groups of people were similar, consisting of healthy physicians and technicians working in the laboratory. Cultures were taken at intervals of from one to three weeks. It is seen that in the first series all but one individual harbored influenza bacilli almost constantly, whereas in the second group there was only one constant and three transient carriers.

In summary, then, it appears that the high incidence of influenza bacilli in the throats of healthy people which was present during and after the pandemic has declined to the "normal" or pre-epidemic figure.

2. *The Seasonal Variation and the Relation to the Incidence of Respiratory Disease.* In each subject studied a careful examination of the throat was made for evidences of abnormality such as diseased tonsils. The history of previous nasal or throat infection or of respiratory disease was also investigated. In brief it may be said that absolutely no relationship between the presence of influenza bacilli, and the occurrence of previous upper air passage infection could be demonstrated in the present observations. It seemed important, also, to determine the seasonal variation, if any existed, in the incidence of influenza bacilli in normal people. Table III shows our cases arranged according to months from August to December. No significant variation appears, although the percentage of positive cultures was somewhat greater during October, November and December than it was in August and September. These results are in accord with those of Winchell and Stillman who found a fairly steady incidence from December 1918 to April 1919, although their figures were much higher than ours.

In summary, then, there is no convincing evidence that the incidence of influenza bacilli in healthy people varies with the incidence of mild respiratory infection.

TABLE III.

Seasonal Incidence of Bacilli in Healthy People

		Positive	Negative	Per cent Positive
August	1921	8	46	14.8
September	1921	5	40	11.1
October	1921	16	54	23.0
November	1921	10	42	19.2
December	1921	10	52	16.0

3. *Carriers.* In the present studies our interest in influenza bacillus carriers has centered in the question whether any law governing the adaptation of these organisms to growth on human mucous membranes could be deduced. The literature considered from this point of view is meager.

(a) *Carriers Associated with Chronic Disease.* As pointed out above, the influenza bacillus is often found in chronic pulmonary lesions. Lord¹⁰ and Luetscher⁹ report its presence in a variety of conditions; Boggs⁴⁷ pointed out its frequency in bronchiectasis; and Miller⁴⁸ has stressed its presence in chronic basilar non-tuberculous infections.

(b) *Carrier States Following Experimental Inoculation of Influenza Bacilli in Man.* Wahl, White and Lyall⁴⁹ applied a saline emulsion of influenza bacilli from epidemic cases to the mucous membrane of the nares and pharynx of five healthy men. They were unable to recover the organisms from the nose after forty-eight hours except in one case, but found them present in the naso-pharynx for two weeks or longer. In some cases the bacilli disappeared after a few days to reappear later. There is doubt, therefore, as to the identity of the strains recovered on successive cultures. Sellards and Sturm,²⁶ following inoculations into the nose and throat, recovered no influenza bacilli after three days. Bloomfield⁵⁰ used three strains isolated from healthy men. In twelve experiments in which inoculations were made on various areas of the upper air passages the strain introduced could not be recovered after forty-eight hours, but in five cases Pfeiffer bacilli isolated later than twenty-four hours were found to differ from the experimental strains. Davis⁵¹ found influenza bacilli in the throat of a volunteer four weeks after inoculation. Cecil and Steffen,¹⁷ after experimental inoculations with supposedly virulent strains, noted a persistence of bacilli lasting from six hours to two months in various cases. The identity of the strains was checked by agglutination tests. Park and Cooper¹⁸ studied four cases of accidental inoculation with influenza bacilli. In one instance no carrier state was set up, in the others bacilli were present after 4, 28, and 68 days respectively.

(c) *Spontaneous Carrier States in Normal (Clinically Healthy) People.* Povitzky and Denny⁵⁰ found that influenza bacilli persisted in the throat for lengths of time up to one year. Serological tests showed that the strain changed in some cases, while in others the same organism persisted. Bloomfield⁴⁴ found influenza bacilli

repeatedly during a period of about three months in nine individuals. No proof of the identity of strains from a given carrier was obtained. Winchell and Stillman⁴² made careful studies on a group of people during the winter of 1918-19. Cultures were made every month on the same eighty-five individuals. Fifteen never yielded a positive result, nine of thirty-five positive on the first examination never showed influenza bacilli later, six of the eighty-five were positive for five months and thirteen for four months. Unfortunately, it was not determined whether the same strains persisted or whether new ones appeared from time to time.

In summary, then, the literature brings out the following points in regard to carriers of influenza bacilli.

(1) Influenza bacilli may be carried for prolonged and indefinite periods of time by individuals affected with chronic respiratory disease—bronchitis, bronchiectasis, lung abscess, etc. In this case the organisms seem to live and multiply in the focus of infection whence they are discharged.

(2) Normal (clinically well) people without any recent history of respiratory disease may harbor influenza bacilli in the throat. While the observations on record are few, it appears that such a carrier state may be brief or prolonged, and that in some cases the same strain of organism may be present for months or a year.

(3) Great variations have followed the experimental introduction of influenza bacilli in man. In some cases the organisms were promptly eliminated, in others a carrier state was set up lasting for weeks or even months. The situation is complicated by the doubtful relation of some of the cases to acute respiratory infection, by the fact that no exact observations were made on the location of the organisms in the upper air passages, and by the fact that in some cases old laboratory strains and in others freshly isolated human strains were used.

Furthermore, since the observations in normals were made during or shortly following the epidemic, at a time when the behavior of influenza bacilli was a variable affair, no conclusion can be drawn from them as to the normal adaptive power of *B. influenza* to human mucous membranes.

TABLE IV.

Results of Serial Cultures in Subject B.

The subject is a physician frequently exposed to respiratory infection. No recent history of colds or sore throat. Throat clear. Small clean tonsils.

Culture Aug. 19, 1921	No influenza bacilli	
Culture Aug. 29, 1921	No influenza bacilli	For past 24 hrs.
Culture Sept. 7, 1921	No influenza bacilli	slight pharyngitis with malaise
Culture Sept. 16, 1921	Innumerable influenza bacilli	Well
Culture Sept. 17, 1921	About 100 cols. influenza bacilli	No recent infection
Culture Sept. 20, 1921	About 100 cols. influenza bacilli	

Culture Sept. 30, 1921	No influenza bacilli
Culture Oct. 4, 1921	No influenza bacilli
Culture Oct. 17, 1921	No influenza bacilli
Culture Nov. 9, 1921	No influenza bacilli
Culture Dec. 12, 1921	No influenza bacilli

Comment.—It is seen that B. developed a transient carrier state lasting only a few days and apparently quite unrelated to any external factor.

TABLE V.

Results of Serial Cultures in Subject H.

The subject is a physician frequently exposed to respiratory infection. No recent history of cold or sore throat, but he has very large scarred tonsils with plugged crypts.

Culture Oct. 2, 1921	No influenza bacilli	Has been in contact with a patient with chronic bronchitis and a pure culture of the influenza bacillus in sputum
Culture Oct. 4, 1921	50 cols. influenza bacilli	

Culture Oct. 5, 1921	No influenza bacilli
Culture Oct. 17, 1921	No influenza bacilli
Culture Oct. 30, 1921	No influenza bacilli
Culture Nov. 9, 1921	No influenza bacilli
Culture Nov. 15, 1921	No influenza bacilli
Culture Dec. 12, 1921	No influenza bacilli

Comment.—An example of a very transient carrier state, the source of the influenza bacilli was possibly the patient referred to above. Two cultures were made from material aspirated from the crypts of H's tonsils. No influenza bacilli were recovered.

TABLE VI.

Results of Serial Cultures in Subject R.

The subject is a physician exposed to respiratory disease. Not subject to frequent sore throats. Very small clean tonsils.

Culture on Sept. 18	No influenza bacilli	
Culture on Sept. 30	No influenza bacilli	Slight pharyngitis—mild constitutional symptoms. Throat slightly red
Culture on Oct. 3	No influenza bacilli	Feels well. Throat clear
Culture on Oct. 17	Innumerable influenza bacilli	Sore throat for 1 day. Throat looks red, patches on tonsils
Culture on Oct. 19	4 cols. influenza bacilli	Outspoken follicular tonsillitis
Culture on Oct. 21	6 cols. influenza bacilli	Temp. 101.°
Culture on Oct. 26	No influenza bacilli	Blood-agar plates show almost pure culture of hemolytic streptococcus.
Culture on Nov. 9	No influenza bacilli	Throat better.
Culture on Nov. 21	No influenza bacilli	Still many hemolytic streptococci
Culture on Dec. 12	No influenza bacilli	Well

Comment.—It is seen that a transient carrier state was present. With the coincident onset of a streptococcus tonsillitis the influenza bacilli rapidly disappeared. Culture from the tonsil crypt showed no influenza bacilli.

TABLE VII.

Duration of Carrier State from Time of First Positive Culture.

Duration	Number of Cases	Remarks
5 months	1	Still positive on last culture
3 months	1	
1 month	1	Still positive on last culture
2 weeks	1	Still positive on last culture
10 days	4	1 case still positive on last culture
7 days	4	1 case still positive on last culture
4 days	2	
2 days	3	
1 day	1	

Our own observations were conducted along two lines. First a set of ten available people was selected. They were all normal clinically and were free from respiratory disease. Cultures were made from time to time over a period of four to five months to see if any members of the group would become carriers and, if so, what the course of the carrier state would be. Cultures from all ten men were negative at first. Seven failed to show influenza bacilli at any time. The other three developed temporary carrier states which were of interest. Their records are presented in Tables IV, V and VI. It is seen that about one third of the group developed a transient infestation with influenza bacilli, emphasizing the probability of an extensive give and take of these organisms among the population at large, independent of respiratory infection and without permanent colonization.

Next a study was made of the persistence and localization of *B. influenzae* in a larger group of carriers. Of the forty-nine subjects who showed positive cultures eighteen were available for further observations. The duration of the carrier state in these people is shown in Table VII. The striking point is the generally brief duration of the carrier state, which lasted ten days or less in twelve of the eighteen cases.

It is a well known fact that carrier states discovered in healthy individuals are usually very persistent. Diphtheria bacilli, as pointed out especially by Guthrie, Moss, and associates,⁵² when once present, usually persist over long periods of time. The same is true of hemolytic streptococcus and Friedländer carriers.⁵³ Furthermore, in these cases it can usually be shown that the foreign organism is harbored in a local focus of diseased tissue such as the tonsil. It seemed of importance, therefore, to determine in the case of influenza bacillus carriers whether any relationship existed between the duration of the carrier state and the localization of the organisms in the throat. A series of differential cultures was therefore made the results of which are shown in Tables VIII–XIII.

TABLE VIII.

Differential Cultures from Carrier R. This subject is a healthy man. He says that his tonsils have never been removed, but little tonsillar tissue is visible. The throat is clear.

No. cols. B. influ.	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
	Aug. 18, 1921	∞	∞	∞	0
	Aug. 29, 1921	many	many	many	0
	Sept. 19, 1921	0	40	50	0
	Sept. 30, 1921	0	0	0	0
	Oct. 4, 1921	0	6	0	0
	Oct. 19, 1921	100	2	2	0
	Nov. 4, 1921	0	0	20	0
	Nov. 11, 1921	0	∞	∞	0
	Dec. 14, 1921	many	many	a few	0

Comment.—No tests were made to prove the identity of influenza bacilli recovered on successive cultures. It is seen, however, that there was no sharp localization of the organisms at any special site.

TABLE IX.

Differential cultures from Carrier V. Healthy man. Huge globular tonsils almost meeting in midline. No acute process at present.

No. cols. B. influ.	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
	Nov. 7, 1921	300	0	a few	0
	Nov. 8, 1921	200	200	20	0
	Nov. 9, 1921	∞	100	∞	0
	Nov. 11, 1921	∞	100	35	1

Comment.—No localization made out.

TABLE X.

Differential Cultures in Carrier G. Patient has acute nephritis. Tonsils removed two weeks ago. Still a small raw area on the right, but throat is clear.

No. cols. B. influ.	Site of Culture	Rt. Tonsil (fossa)	Lt. Tonsil (fossa)	Pharynx	Soft Pal.	Tongue
	Nov. 5	0	0	∞	0	0
	Nov. 7	0	0	150	0	0
	Nov. 9	0	0	100	0	0
	Nov. 11	0	0	0	0	0
	Nov. 14	0	0	20	0	0
	Nov. 18	0	0	0	0	0
	Nov. 28	0	0	0	0	0

Comment.—Although the carrier state was of relatively brief duration, the organisms were confined to the pharyngeal wall.

TABLE XI.

Differential cultures in Carrier H. Multiple neuritis. Not subject to sore throats. No recent respiratory infection. Very small clean tonsils. Nose and throat examination by specialist, negative.

No. cols. B. influ.	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
	Nov. 5	0	0	∞	0
	Nov. 7	0	0	200	0
	Nov. 9	0	0	0	0
	Nov. 11	0	0	50	0
	Nov. 14	0	0	∞	0
	Nov. 18	0	0	0	0
	Dec. 8	0	0	0	0
	Dec. 12	0	0	0	0

Comment.—A brief carrier state with the organisms confined apparently to the pharynx.

TABLE XII.

Differential cultures in Carrier Gr. Normal man. Rather large globular but clean tonsils. Not subject to respiratory infections.

No. cols. B. influ.	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
	Aug. 22	many	many	many	0
	Aug. 25	40	0	2	0
	Sept. 5	0	0	0	0
	Sept. 12	0	0	0	0
	Sept. 17	0	0	0	0

TABLE XIII.

Differential cultures in Carrier N. Healthy man. Small ragged tonsils. Not subject to sore throats. No recent respiratory infection.

No. cols. B. influ.	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
	Aug. 20	200	300	0	0
	Aug. 26	150	6	0	0
	Sept. 7	200	∞	0	0
	Oct. 19	many	many	many	0
	Nov. 20	0	0	0	0
	Nov. 26	0	0	0	0

It is seen that in the case of carrier states of varying lengths no definite law of localization can be derived. The organisms may be confined to one or another area or may vary from time to time in their distribution. A check on the above findings was provided by making a series of twelve cultures from material aspirated from the depths of tonsil crypts. In two cases a few influenza bacilli were found. These findings are therefore in contrast to the conditions obtaining in carriers of streptococci (hemolytic), diphtheria bacilli, Friedländer bacilli and others, where one usually deals with a persistent localization in the depths of infected lymphoid tissue.

DISCUSSION

The main results of the present work may be briefly summarized as follows. In the first place it appears that the general incidence of influenza bacilli in the throats of healthy people has declined greatly since the time of the pandemic, the present figure being comparable to that which existed before the wave of influenza in 1918. Furthermore, there seems to be no striking relationship to the season of the year or to the prevalence of minor respiratory infections. An outstanding feature of the carrier state in the individuals we have observed was its usual relatively brief duration and the absence of evidence in the case of healthy people of localization of influenza bacilli in a localized area of diseased tissue such as the tonsil.

A few words may now be said in regard to the general bearings of our findings on the broader question of bacterial adaptation to growth on respiratory mucous membranes. In another place⁵⁴ it was pointed out that criteria of test-tube growth do not serve to explain the presence in the throat of certain organisms and the absence of others, but that one must assume subtle biological adaptations, the exact chemistry of which is as yet obscure. It was shown that bacteria such as green-producing streptococci possess a high degree of inherent adaptation, as indicated by their early and constant appearance in the throats of infants and their almost constant presence in adults as well. On the other hand, other organisms such as *B. coli* seem to have a total lack of adaptation to growth in these regions. Furthermore, evidence was presented that still other organisms possess partial degrees of adaptation,⁵⁵ occurring frequently but not constantly. The hemolytic influenza bacilli, for example,—which as far as we know play a negligible

part in human pathology—are found in from 30 to 50 per cent of throats of healthy people, as a rule unassociated with any focal infection but seemingly growing free on the mucous membranes. The question which now logically arises is whether the adaptation of organisms to growth on mucous membranes may not undergo waves of fluctuation depending on a variety of factors. Many observations in the literature give hints that this may be so. Hemolytic streptococci, while frequently present in focal infections in diseased tonsils,⁵⁶ are not apt to be found on the free surfaces of the normal mucous membranes.⁵⁷ When experimentally introduced into the throat, they either disappear⁵⁸ rapidly or set up a tonsillitis in association with which they can be recovered in culture.^{17,49} It is clear, then, that the present average adaptation of hemolytic streptococci to free growth on normal mucous membranes is very low and that a local infection is necessary for permanent colonization. During the war, however, at a time when malignant streptococcus disease was epidemic, the passage of hemolytic streptococci through many people seems to have altered the organisms in such a way that a much higher adaptation to growth on mucous membranes existed. Hence these organisms were found in a high percentage of throat cultures from normal people⁵⁹ in contact with streptococcus disease. Even here, however, the carrier state seems to have been temporary, and after the epidemic it promptly reverted to the average conditions mentioned above. One may cite also in this connection the frequent association of hemolytic streptococci with scarlet fever and other acute infections. Similar considerations seem to apply to the meningococci. In inter-epidemic times they are practically never found in healthy people and then probably only in local foci of infection, whereas during epidemics the carrier rate may be as high as twelve to eighteen per cent.⁶⁰ Clearly an alteration has temporarily taken place which leads to a greater degree of adaptation to growth on mucous membranes.

The influenza bacilli, however, present by far the most picturesque example of such changes in adaptation. The normal state of affairs in regard to the adaptation of these organisms seems to be that they show a widespread association with chronic focal infections in the respiratory tract. There is no evidence that they have more than a moderate and partial degree of adaptation to free growth on normal mucous membranes. A variety of acute infections, however, alter the soil in such a way that influenza bacilli flare up as it were and temporarily become adapted to widespread growth in the affected individuals. This has been shown to be the case in scarlet fever (39%) by Jehle,⁶¹ in diphtheria (60%) by Jehle, in varicella (100%) by Jehle, and in measles (78%) by Jehle, (47%) by Susswein, (33%) by Wollstein, (56%) by Davis and (79%) by Sellards. The observations of Sellards²⁶ are of particular importance, because exami-

nations were made at various stages of the disease. Influenza bacilli were found in seventy-nine per cent of the cases during the eruptive stage, in twenty-four per cent ten to fourteen days after eruption, in twenty per cent twenty to twenty-five days after eruption, and in no case forty days after the eruption. In these diseases, therefore, the adaptation is temporary and apparently not of a degree high enough to allow growth to extend generally to the mucous membranes of healthy people.

It is of interest that the mild epidemic grippé and the common cold are relatively inadequate to produce such marked alterations in adaptation.⁶² Epidemic influenza, on the other hand, produces this effect to a maximal degree. The result was, as has been pointed out, not only an almost uniform incidence in cases of the disease but in the normal population as well. This change persisted for at least two years after the epidemic, but now, three years after the epidemic, conditions, as indicated by our figures, seem to have returned to the normal average. In summary, then, we have in the case of the influenza bacillus group, organisms which show a marked variation at various times in their adaptation to growth on human mucous membranes. A variety of altered conditions, especially acute infectious diseases, may produce this change but epidemic influenza does so to an extreme degree.

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THE INTERCOLUMNAR TUBERCLE, AN UNDESCRIBED AREA IN THE ANTERIOR WALL OF THE THIRD VENTRICLE

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In a recent paper,¹ it was pointed out that the area postrema of the fourth ventricle became deeply colored in animals injected with trypan blue. It was supposed that this structure was the only intrinsic portion of the brain which took such a stain. A more minute examination of the brains of some of these animals, however, revealed the presence of a small tubercle of tissue, similar to that composing the area postrema, in the anterior wall of the third ventricle. But while the area postrema were probably known to Stilling, and were well described by Retzius, the analogous area in the third ventricle has apparently been overlooked by anatomists.

This area is situated between the anterior pillars of the fornix, about on a level with the superior border of the foramina of Monro in the species studied, and below the juncture of the two choroid plexuses of the lateral ventricles. It might therefore appropriately be called the *intercolumnar tubercle*. It may be exposed, in the animals observed, by an accurate frontal section passing just in front of the optic chiasm. In the fresh unstained specimen, it can usually be recognized without difficulty as a pearly, grayish, translucent nodule, round or somewhat fusiform and of a slightly variable size, up to one millimeter in diameter and half a millimeter in height. In animals stained with trypan blue or other colloidal dyes (pyrrhol blue) there is some individual variation in depth of staining, but the tubercle is almost always distinctly colored. It may also be brought out by a capillary injection of blood-vessels, or by staining the gross specimen with dilute methylene blue, or certain other diffusible dyes (Bismarck brown, neutral red), when the tubercle takes a distinctly brighter color than the surrounding tissue. In unstained tissues fixed by the ordinary methods, its peculiar pearly appearance is lost, and it can be identified only by its shape and position. It is frequently reduced in size also by fixation. In the human brain, the choroid plexuses must be lifted up or stripped away before it can be seen. It varies but little in size, shape, and position in the various animals studied (rat, guinea-pig, rabbit, cat, dog, monkey and man).

Histologically, the intercolumnar tubercle resembles the area postrema. It is mainly composed of a very loose reticulum of neuroglia cells, which, however, differs markedly in appearance from the fine, regular felt-work seen elsewhere in the brain. The nuclei of the cells composing it are mostly large and elongated, measuring up to 20×10 micra, although smaller and round ones are seen. They stain less deeply with basic dyes than the ordinary spider-

cells, and contain a distinct nucleolus and numerous coarse chromatin granules. The cytoplasm is indefinite in outline, but usually extends two or three micra beyond the nucleus and gives off several short processes. Occasionally it contains large vacuoles. It takes a faintly basic stain. Through it pass wavy fibrils, running from one cell to another; but they are much coarser and longer than the ordinary neuroglia fibrils, less numerous and more widely spaced. Thus, the tissue resembles a mesodermal reticulum in structure more than it does neuroglia, but none of the fibers are stained by picrocarmine or anilin blue, while nucleus, cytoplasm and fibrils are stained blue by Mallory's phosphotungstic hematoxylin. In addition, the relation of the tissue to the ependyma and to the remainder of the brain speaks against a possible mesodermal origin.

In the human brain, the tubercle contains large, triangular cells which resemble nerve-cells. The nucleus is pale, round or oval, about 20 micra in diameter, and has a large, dark, eccentric nucleolus and a few coarse chromatin granules. The cytoplasm takes a faint basic stain, and can be seen to be slightly granular. They are similar to the nerve-cells described by Wilson² in the area postrema.

In the monkey (*Macacus rhesus*) rather smaller cells, still somewhat resembling nerve-cells, are seen in the intercolumnar tubercle and (contrary to a previous report¹) also in the area postrema. They have not been demonstrated in either situation in the lower quadrupeds.

The ependyma over the intercolumnar tubercle is squamous or very low cuboidal, resembling almost a mesothelial lining. It blends gradually with the columnar ependyma over the fornix. It is extremely delicate, and is often injured by even gentle handling.

The tubercle contains many coarse capillaries, with abundant perivascular connective tissue, in which are found the particles of dye in vitally stained animals. The adventitia can also be strikingly demonstrated by Van Gieson's or Mallory's connective-tissue stains. Injections show that these capillaries form a close network, supplied by one of the central branches of the anterior cerebral arteries, and draining into the internal cerebral veins. They do not communicate directly with the choroid plexus, although so close to it. This vascular arrangement is quite analogous to that of the area postrema.¹

The constancy of this structure, its vascularity, its peculiar neuroglia and ependyma, its behavior toward

vital dyes, and (in the human brain) the presence of nerve-cells, all suggest that it has some specialized function. But what this may be, we can at present barely speculate.

It is a pleasure to acknowledge my indebtedness to Dr. George Wislocki for many fruitful suggestions; to Professor Meyer for valuable criticisms; to Dr. George W. Corner for the monkey's brain; to Professor Brödel for assistance with the drawings; and to Professor W. MacCallum for permission to work on this problem, and for help and advice.

SUMMARY

A structure in the anterior wall of the third ventricle of the mammalian brain is described, which appears to be analogous in many ways with the well-known *area postrema* of the fourth ventricle. It is a small prominence, up to one millimeter in diameter, situated between the columns of the fornix, at the level of the upper border of the foramina of Monro, below the juncture of the two lateral choroid plexuses. It is composed of a peculiar loose neuroglia tissue, containing many coarse capillaries, and covered by low ependyma. In the human brain, it contains small nerve cells. The adventitia of the capillaries takes up trypan blue and other vital dyes *intra vitam*. No function can at present be assigned to it. The name *intercolumnar tubercle* is suggested for this structure.

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DESCRIPTION OF FIGURES

1. Brain of human baby at term. (X1.) Slightly oblique section passing through the anterior commissure posterior to the juncture of the lateral choroid plexuses, and anterior to the optic chiasm. The enclosed area is seen slightly enlarged in the photograph (Fig. 2).

- a—intercolumnar tubercle.
- a—anterior columns of the fornix.
- c—choroid plexus.
- d—tip of temporal lobe.

2. Detail of enclosed area in Fig. 1. Slightly enlarged. Untouched photograph.

3. Human brain. Sagittal section through intercolumnar tubercle, low-power photomicrograph. Formaldehyde fixation. Phosphotungstic acid hematoxylin. The intercolumnar tubercle is seen as a low eminence just to the right of the attachment of the choroid plexus, marked by its loose reticular structure and the flatness of its ependyma. The wide capillaries are surrounded by a generous amount of adventitia. A few nerve-cells are seen. The enclosed area is enlarged in Fig. 4.

4. Detail of enclosed area in Fig. 3. High-power camera lucida drawing. The characteristic flat ependyma is seen at the left, blending with the usual columnar ependyma at the right of the picture. The looseness of the mesh-work, and the size and shape of the neuroglia cells is shown. The shrunken adventitia of a large capillary is seen near the lower left-hand corner, and a smaller one in the right corner. Several nerve-cells are illustrated.

5. Brain of dog. Slightly enlarged. Blood vessels injected with india ink, cleared in oil of wintergreen. Frontal section passing through optic chiasm. Lens enlargement. The area enclosed by lines, containing the intercolumnar tubercle, is enlarged in Fig. 6.

6. Detail of enclosed area in Fig. 5. The intercolumnar tubercle appears as a small vascular mound near the center of the drawing.

PSEUDOMYXOMA PERITONEI

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INTRODUCTORY

In pseudomyxoma peritonei all or a part of the peritoneal surface is covered, and the body cavity, locally or generally, filled with gelatinous material, while the peritoneum itself usually shows some roughening and thickening. The jelly-like substance may occupy only part of the pelvis or one iliac fossa; or, in the extreme cases, the abdomen may be greatly distended by the semi-fluid material which fills practically all available space within the peritoneum. Sometimes the peritoneum is only moderately injected, at other times it may be enormously thickened, in some cases almost to a centimeter. Whitish bodies, pin-head to pea-sized, may be found, either as excrescences from the peritoneum or free in the gelatinous secretion; in these bodies there may be glandular masses, apparently of the nature of metastases.

HISTORICAL

The first important modern literature on the subject of pseudomyxoma peritonei dates back to 1884, when Werth described the condition and declared that it always has its origin in the so called pseudomyxomatous tumors of the ovary. This author averred, moreover, that the peritoneum itself shows only a foreign-body reaction. Ols hausen and Pfannenstiël, however, a little later predicated actual peritoneal metastases or implantations, saying that the extruded gelatinous material includes epithelial cells which continue to multiply and produce more of the same material. Wendeler, in 1896, follows Werth and speaks of a chronic productive or adhesive peritonitis, resulting from the rupture of a gelatinous cyst of the ovary. Fränkel, in 1901, made a notable contribution, reviewing the entire subject, and then describing two cases of his own.

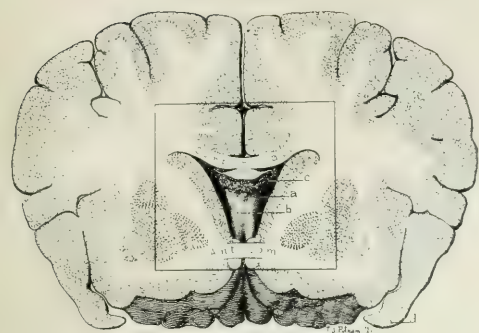


FIG. I.



FIG. II.

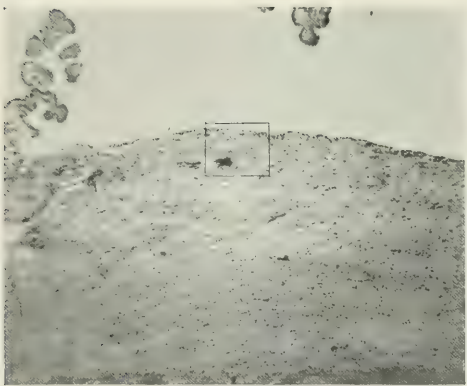


FIG. III.

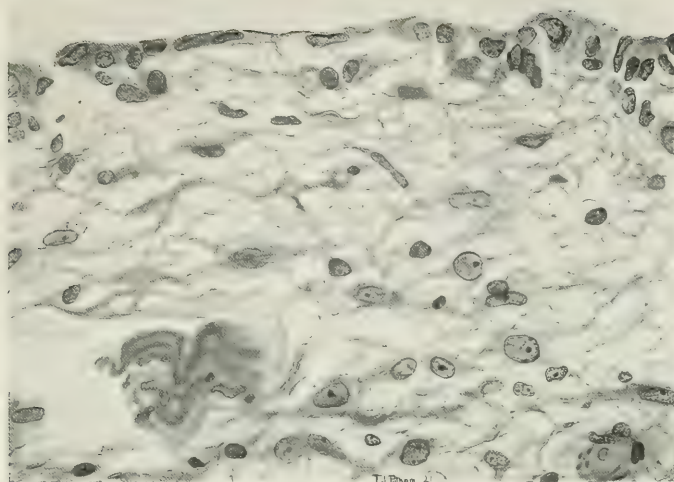


FIG. IV.



FIG. V.

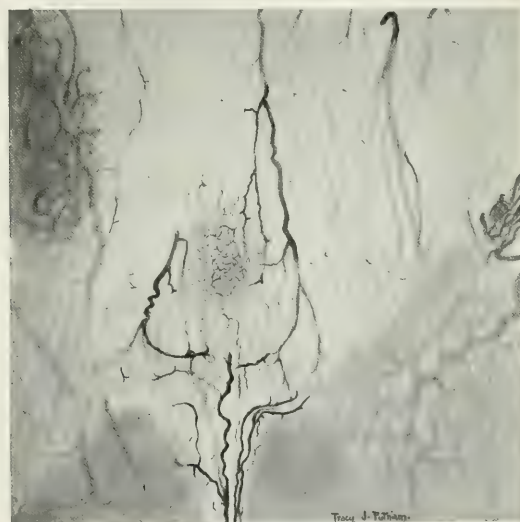


FIG. VI.

Fränkel's first case was that of a woman with a typical pseudomucinous cystadenoma of the ovary, which had ruptured. On the visceral peritoneum he found small implantation cysts, lined with epithelium a little lower than but otherwise similar to that in the ovarian tumor, and evidently actively producing the same pseudomucin.

The second case was that of a man who at autopsy was found to have a cystic appendix which had ruptured and from which had exuded gelatinous material. This material filled the pelvis and lower abdomen. Fränkel's report of this case marks the beginning of our knowledge of peritoneal pseudomyxoma in the male.

PSEUDOMYXOMA PERITONEI EX PROCESSU VERMIFORMI

Since Fränkel's case of pseudomyxomatosis of the peritoneum originating in the appendix, a number of other instances have been reported. Wilson, in 1912, mentions twelve cases and adds two more; since then a few additional reports have appeared, so that the occurrence of the condition is well established. Dodge, who collected from the literature a large number of cases of cystic appendix, found among them fourteen instances of pseudomyxoma peritonei caused by rupture or leaking of a pseudomucinous cyst of the appendix. In Seelig's recent article is a clear discussion of pseudomyxoma of the peritoneum in the male, which, he says, always arises from the appendix. On this point there is a difference of opinion, some holding that certain male cases are associated with a normal appendix. The actual origin in these cases is unexplained, but by some is assumed to be in the retroperitoneal tissues.

According to Seelig, epithelial cells in the exudate have been demonstrated; but the course of the disease does not seem to be dependent on the presence or absence of these cells. In his case the appendix was not seen, and presumably had been removed at an earlier operation. Cysts in the exudate were numerous, some lined with epithelial cells, some with mere shadows of cells. The spleen was very extensively invaded and the entire course was malignant. Lewis also shows that in her case there were many peritoneal cysts; and she further gives an excellent presentation of the chemistry of the condition.

RELATIVE IMPORTANCE OF APPENDIX AND OVARY AS SOURCE.

Eden and others have described a very few instances, perhaps less than half a dozen, in which pseudomyxoma peritonei had undoubtedly originated from an ovarian cyst, but in which there co-existed an appendiceal cyst with gelatinous contents. In those cases in which the appendix and the ovary are both involved, it is not possible always to say offhand which is primary and responsible for the peritoneal condition.

Interesting suggestions have been made, pointing to a distinction between the peritoneal conditions caused by the appendix and the ovary, respectively. Fränkel in his

careful postmortem studies of the peritoneum states that, when the masses of gelatinous material come from the ovary, they cause little reaction, and that at most they may be encapsulated by a very light membrane. He showed metastatic or implantation cysts, actively secreting. When the appendix is the source, he says, the peritoneum shows definite roughening, with organization of the foreign body and overgrowth by endothelium. Furthermore, no cystic metastases are found. Some authors however, with Seelig, state that epithelial cells may be extruded from the appendix also, and may at times multiply.

Fränkel assumed that the difference in the effect upon the peritoneum depends upon a chemical difference between the appendiceal and the ovarian secretions. Trotter says that in the appendix cases the jelly contains mucin, in the ovarian cases pseudomucin. In the literature, where the ovarian content has been studied at all, it seems to have been pseudomucin. As to the appendix cysts, in the large, almost complete series collected by Dodge, in only six cases was the chemical nature of the contents mentioned. In three cases the contents are reported as mucin, in two as pseudomucin and in one as colloid.

Certain it is that in the great majority of cases in which both the ovary and the appendix are the seat of pseudomyxomatous degeneration the ovary, and not the appendix, is responsible for the peritoneal involvement—as in the two cases reported below. Taking all cases of pseudomyxoma of the peritoneum in females, it is of course commonly found that the ovary itself is the only possible source. Pseudomucinous ovarian cystadenoma is relatively common. Wilson says that it forms from two to four per cent of all ovarian cysts. Appendiceal cysts, on the other hand, are relatively uncommon. Dodge, in 1916, collected 142 cases; I have found in the literature twelve others; and in the records of the Gynecological Department of The Johns Hopkins Hospital I have discovered nine more. From necropsy statistics Castle gives the incidence as 0.2 per cent, Corning as 0.5 per cent, Kelly and Hurdon as 0.42 per cent, Elbe as 0.5 per cent and Ribbert as a little over one per cent.

When appendiceal cysts do occur, however, they are most often of the pseudomucinous variety. Of the 142 cases reported by Dodge, in 81 the contents are described, and in 59 of these they were of a mucoid, colloid, gelatinous or pseudomucinous nature. In three of the nine cases found in the Hopkins gynecological records the appendix contained gelatinous material.

The evidence indicates, therefore, that in a given case of pseudomyxoma peritonei in a female the most likely source is in the ovary, especially if the peritoneal condition is extensive. The collection of gelatinous material from a cystic appendix usually is confined to the pelvis; although some cysts of the appendix form very large

tumors, and if ruptured might set free considerable amounts of secretion. Where a cystic ovary and a cystic appendix co-exist, the discovery of a point of rupture in one or the other organ will of course be decisive. When no point of rupture or perforation is evident, or when both ovary and appendix have been opened, a chemical examination of the exudate, as well as a histological examination of the peritoneum, may be helpful.

TWO CASES OF CYSTIC APPENDIX ASSOCIATED WITH
PSEUDOMYXOMA PERITONEI ARISING
FROM AN OVARIAN CYST

CASE 1.—*Gyn. Path. No. 23008 from the Church Home and Infirmary.* A white woman, 73 years of age, entered the hospital in April, 1917, complaining of marked abdominal enlargement. She had had the ordinary diseases of childhood, but since then had enjoyed good health until the present illness. She had married at 21 but had never been pregnant. The periods had always been irregular; the menopause had occurred at 45. The present illness had begun nine months before admission with loss of weight. For two months there had been slight vaginal bleeding, with remissions of a few days. Gastric symptoms had become marked only recently, with acid regurgitation and occasional vomiting. One month before admission the patient had noticed a lump in the lower right quadrant of the abdomen, and this swelling since then had increased rapidly in size.

Examination revealed a growth strongly suggestive of a cyst, evidently arising from the pelvis and partly filling the lower abdomen.

Operation by Dr. Cullen on April 25, 1917.—When the abdomen was opened, there was at once encountered a lumpy fluid reminding one of that found where colloid carcinoma exists. The cyst was multilocular in type and had very thin walls. It was removed with some difficulty. There was no evidence of metastasis. The appendix near its tip was one centimeter in diameter; it was removed.

Subsequent History.—Recovery from operation was uneventful. In December, 1917, the patient was reported well. By January, 1918, she had changed greatly; she had lost much flesh, was suffering from severe gastric symptoms, and the abdomen was full and tight. A little later she died.

Pathological Note (Dr. Wynne): The specimen consists of an appendix and an ovarian cyst. The appendix is 3.5 cm. long and 13.5 mm. in diameter. The surface is covered with adhesions. On section the lumen is greatly dilated, measuring one centimeter across. It contains a curious substance, which has the general appearance of a papillomatous mass embedded in gelatin. The ovarian cyst measures roughly 25 by 20 by 15 cm. It is subdivided by many thick partitions of grayish tissue. Many of the loculi are ruptured and a thick, yellow, gelatinous material exudes.

Microscopic Examination. Ovary.—The cyst walls have a compact, fairly vascular stroma, thrown up in irregular polypoid folds. The lining is of cylindrical epithelium with many goblet cells. The amorphous contents of the cyst stain with eosin. There are some hemorrhagic areas, with beginning organization. In some places the stroma is infiltrated with leucocytes, chiefly mononuclears.

Appendix.—The serosa is thickened and congested in places. No abnormality is seen in the muscularis. The mucosa is of very high cylindrical epithelium, most of the cells being of the goblet type. The mucosa is heaped up in papillomatous masses which nearly fill the lumen. The submucosa can be followed into the polypoid projections. No evidence of invasion or penetration of the muscle is seen.

Chemical.—The gelatinous material from the ovary, the appendix and the peritoneal cavity, when examined, had been in formalin, which may have affected its reactions. It could not be definitely identified as paramucin, pseudomucin or colloid. It was insoluble in alkali, acetic acid and mineral acid.

CASE 2.—*Gyn. Path. No. 23580, Gyn. No. 23650.*—A married colored woman, aged 39 years, was admitted November 23, 1917, complaining of swelling of the abdomen and backache. The family history was unimportant. Menstruation had usually been regular, the last period having occurred in September, the preceding one in August. The last three periods had been very scanty. Prior to October there had been no missed periods except during pregnancy. No metrorrhagia had been noted. The patient had had four normal pregnancies, and no miscarriages. The present illness had begun four months before admission with epigastric pain at first only on exertion, later unassociated with it. For two or three months the abdomen had been becoming larger and increasing dyspnea and orthopnea had followed.

Examination showed a temperature of 97°, pulse 84, respirations 40. The woman was in great distress, vomiting, dyspneic and absolutely orthopneic. The abdomen was tremendously distended, but evidences of collateral circulation were only fairly distinct. A definite fluid wave was obtained. Pelvic examination showed simply bulging in the fornices and cul-de-sac. The lungs were clear except for signs of compression from below. The heart was somewhat dilated and there was a to and fro murmur, loudest at the tricuspid area. The blood pressure was moderately elevated, the vessels thickened. The extremities showed marked brawny edema. The urine contained a trace of albumin and a few granular casts. The Wassermann reaction later proved negative.

Dr. Wynne decided that immediate relief for the dyspnea was necessary, and under cocaine made a small incision below the umbilicus. The peritoneum was not recognized, and a cyst was cut into. There followed a gush of bloody fluid and gelatinous material, which was of a very clear light yellow color. This was let out gradually until about ten liters had been evacuated. The major operation was performed next day. A large amount of bloody fluid was found in the abdomen, with the same sort of gelatinous material evacuated the preceding day. This was removed and a cystic mass of considerable size brought up and found to have ruptured anteriorly. The walls were very thin and friable. This tumor had originated on the left side. A second ovarian cyst on the right side involved the broad ligament. The uterus was of normal size, with a few implantation nodules over it. In the cul-de-sac also were a number of implantations. The entire sigmoid, ascending and descending colon were covered by the gelatinous material, which was very adherent. The bowel walls were greatly thickened and edematous. No nodules were seen in the omentum. The parietal peritoneum was thickened and rough, anteriorly about 4 mm. thick, very friable and in places marked by reddish excrescences. The appendix was swollen, larger at the tip than at the base. The uterus, adnexa and appendix were removed.

Subsequent History.—Recovery from the operation was smooth. On March 12, 1918, the patient seemed to be well except for symptoms of artificial menopause.

Pathological Note.—The right ovary, the tubes and the uterus are all in one mass. The uterus is normal. The right tube runs as a thick ridge over the top of the main mass, which represents the right ovary. This mass is irregular in shape, about 20 by 15 by 15 cm. It has a thick fibrous wall and many compartments, some of which have ruptured and collapsed. The contents are of a gelatinous nature. The walls of the locules are thin, hard and fibrous. Adherent to the inner walls are small masses, some of which are hemorrhagic or suggest vascularization. In a

few places are fine, whitish, papillomatous projections. No normal ovarian tissue is seen. The second part of the specimen represents the left ovary, and measures 15 by 10 by 8 cm. The gross picture of the tumor is essentially the same as that in the other ovary. The appendix is 3.5 cm. long, 1.75 cm. in diameter. It is ovoid in shape, feels cystic and has a definite constriction at the base. The surface is rough, opaque and a little injected. The walls are intact. On section the lumen is dilated to 8 or 9 mm. in diameter. The walls are firm, their thickness ranging from 2 to 6 mm. Near the base the lumen appears to be obliterated. Definite muscular layers can be distinguished and there seems to be an intact mucosa. No smaller cysts can be seen in the walls of the appendix. The lumen contains nothing but clear fluid.

Microscopic Examination. Ovaries.—The cyst walls consist of a dense stroma in heavy masses, compact and cellular. The epithelial lining is flattened, of cuboidal type, with a few goblet cells. There is a slight tendency to the formation of papillomatous projections.

Appendix.—The mucosa is flattened and there is but little infolding. In some transverse sections the mucosa is compressed, the muscularis normal, the lumen greatly distended. In other sections the submucosa is greatly thickened by inflammatory tissue and the lumen is much reduced. In places the submucous and muscular coats are infiltrated with many plasma cells and a few cells of the polymorphonuclear type.

Chemical.—The contents of the ovarian cysts and the abdominal jelly gave definite reactions for pseudomucin.

As to the apparent implantations in the peritoneum, unfortunately no specimens were taken at the time of operation.

SUMMARY AND COMMENT

In the male pseudomyxoma peritonei nearly always occurs from the rupture of a gelatinous cyst of the appendix. In rare cases it may possibly arise elsewhere, *e. g.*, in the retroperitoneal tissues.

In the female this condition may be due either to ovarian or to appendiceal disease. Sometimes both ovary and appendix are affected, and it may be difficult to say which is responsible for the peritoneal involvement. In such cases the chemical nature of the secretion may be valuable evidence.

In the first case here reported the jelly-like material of the abdomen obviously came from the ovary, as the appendix was unruptured. The appendiceal cyst is of the variety shown by Dodge's figures to be the most common, that is, it contains gelatinous material. It is, however, associated with a marked papillomatous condition of the mucosa.

The second case also is one of an ovarian cyst ruptur-

ing into the peritoneal cavity. The cyst of the appendix, a simple hydroph, is probably only coincident, and is a less frequent form of cyst than the first.

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HISTAMINE AS AN INFLAMMATORY AGENT

By WILLIAM BLOOM

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This research was undertaken with the view to determine whether histamine is positively chemotactic and whether it can cause inflammation. The author wishes to express his deep appreciation to Dr. Arnold Rich of the Department of Pathology for his sympathetic interest,

aid, and advice in the carrying out of this research.

The process of inflammation has been studied in detail both histologically and *in vivo* so that the progression of the pathological process is known quite clearly; but we are still entirely ignorant of the substance, or substances,

that call forth this rather stereotyped reaction to injury on the part of the tissues. The beginning of the inflammatory process is the dilatation of the capillaries and smallest arterioles and venules in the affected area; this is followed ordinarily by an exudation of fluid from the widened vessels and the migration of leucocytes into the inflamed area. Inflammation proceeds in fundamentally the same manner whether it be provoked by bacteria or by necrosis of cells in the absence of bacteria; so that one feels that perhaps the inflammatory inciting substance or substances may be something liberated from damaged tissue cells rather than extraneous bacterial toxins. Indeed, Massart and Bordet¹ have shown that dead tissue extracts are positively chemotactic, although the actual chemotactic substance has not as yet been isolated. The substance or combination of substances that causes inflammation must satisfy the following demands: (a) it must be a capillary poison, (b) it must be present in all tissues, (c) it must be liberated through trauma and bacterial action on proteins, and (d) it must attract leucocytes to the focus of injury.

The work of Dale, Laidlaw and Richards² has directed considerable attention to histamine (B—iminazolyethylamine) because of the dramatic effects upon the circulation which minute amounts of the substance produce, and because of the striking similarity between surgical shock and the condition induced by the intravenous injection of histamine. Dale and his co-workers reached the conclusion, through indirect methods, that the effects of histamine on the circulation are the results of its action as a capillary poison—causing dilatation and increased permeability of the capillaries. The capillary dilator effect of histamine was not observed directly by these workers; but recently Hooker,³ studying the skin capillaries *in vivo*, has watched capillary dilatation occur under the influence of histamine, and Rich⁴ has demonstrated histologically that the capillaries of the omentum (of the cat), and the smallest arterioles and venules as well, are markedly dilated, and that previously closed capillaries are opened up during both histamine shock and local applications of histamine. Abel and his co-workers⁵ have shown that histamine is a constituent of all animal tissues. The work of Bayliss⁶ and Cannon⁷ indicates that crushing the leg muscles of a cat will produce in the animal a condition that resembles histamine shock. Ackerman⁸ and Mellanby and Twort⁹ have shown that histamine is formed by the action of bacteria on histidine, an amino acid constituent of all proteins. In view of these results it was thought that, if it could be shown that histamine was positively chemotactic, a possible explanation of inflammation would result.

It was realized in approaching this problem that some means would have to be found to localize the histamine in the tissues; since many highly inflammatory substances produce no reaction when injected intra-

venously (Adami).¹⁰ It is because of this that it was thought that the failure of Paul¹¹ to produce leucocytosis by intravenous injection of histamine in the rabbit was inconclusive.

METHODS

Because of its susceptibility to histamine the cat was used in the following experiments. Hoffman, La Roche histamine dihydrochloride was used in all experiments.

I. A large number of cats were injected intraperitoneally with 3 mg. of histamine in 10 c.c. of physiological saline and an equal number of cats were injected with saline alone as controls. The animals were killed with ether at ten, fifteen, thirty minutes, and one, two, four, six and eighteen hours after injection. Under aseptic conditions the peritoneal cavity in these animals was opened and inspected for fluid. When any fluid was present, it was collected and tested for free cells and for its ability to coagulate, and cultured to exclude the possibility of bacterial contamination. These experiments were performed to determine whether, if histamine be a capillary poison, there would be a difference in the transudate caused by it and by saline.

II. To study the more locally inflammatory action of histamine, the following subcutaneous injections in the ear were made. Sufficient histamine, 1:100, in physiological saline, was injected to raise a bleb 1 cm. in diameter. Control injections were made with physiological saline alone. The blebs would disappear in one or two hours. Forty, eighty, one hundred and ten minutes and twenty hours after the injections had been made the cats were killed with ether, the ear removed, fixed in a formaldehyde-Zenker fluid and then sectioned after imbedding in paraffin. The sections were stained with hematoxylin and eosin and with MacCallum's bacterial stain. In order to localize the histamine for longer periods of time in the subcutaneous tissues, 20 mg. of histamine-hydrochloride crystals suspended in 0.5 c.c. of olive oil were injected subcutaneously in the ear. Control injections were made with croton oil, 1:200 in olive oil. In these experiments the same technique for preparing the sections was employed as that used for the saline injections. Intramuscular injections of paraffin oil and of paraffin oil containing 20 mg. of histamine crystals in suspension were made.

III. It was thought desirable, however, to keep the histamine localized for longer periods of time than the previous methods permitted. Accordingly, collodion capsules containing histamine 1:100 in physiological saline and control capsules containing physiological saline alone were placed, with as little injury to the tissues as possible, between the external and internal oblique abdominal muscles of the cat. Other control experiments were made with capsules containing 5% lactic acid, a negatively chemotactic substance, and with capsules containing croton oil, a highly inflammatory substance. The capsules

were allowed to remain in the animals for periods of time varying from eighteen to twenty-two hours and after the animals had been killed with ether the capsules were removed with the contiguous muscle and treated histologically as in I.

The collodion capsules were made by drawing a viscous solution of collodion into glass tubes of 4 mm. bore; the excess collodion was allowed to drain out, and the thin-walled, hollow, collodion cylinders were extracted from the glass moulds with the aid of cold water, after the ether-alcohol mixture had evaporated. The tubes were then filled with the substances to be experimented with and were tied off at the desired lengths with silk thread. To prevent the collapse of the capsules during autoclaving, it was found necessary to keep them immersed in the same liquids as those that they contained. To test the ability of histamine to dialyze through collodion, capsules containing histamine 1:100 in physiological saline were suspended in physiological saline and after twenty-four hours the dialysate on being injected into a cat produced a typical histamine fall of blood pressure.

IV. To test the purely chemotactic properties of histamine *in vivo*, capillary glass tubes containing histamine 1:100 in physiological saline and control tubes containing physiological saline were buried subcutaneously in the cat. The tubes were sealed at one end to prevent any capillary attraction from drawing in those leucocytes which would respond to the injury produced in planting the tubes. After twenty hours the tubes were removed from the animals and inspected with a microscope to see whether any leucocytes had wandered into the bore—as they should do if histamine is positively chemotactic.

V. Tubes similar to those used in the preceding experiments were surrounded by leucocytes on a warm microscope stage to determine the chemotactic properties of histamine *in vitro*.

RESULTS

I. There was practically no difference between the effects produced by intraperitoneal injections of histamine—3 mg. in 10 c.c. of physiological saline—and of physiological saline alone. The transudate, when present, consisted of a few free cells and a fluid capable of clotting. The greatest amount of fluid was obtained from the peritoneal cavities of those animals killed within one hour after injection. Animals killed after longer intervals than one hour showed very little and those killed at eighteen and twenty hours showed no fluid whatever. It was interesting to note, however, that in those animals which were killed one half hour or less after injection, there was a pink flush in the intestines of only those which had been treated with histamine.

II. Subcutaneous injections of histamine, 1:100 in physiological saline, caused a slight inflammatory reaction that neither qualitatively nor quantitatively differed from the inflammation caused by subcutaneous injection of physiological saline.

Subcutaneous injections of olive oil and of olive oil with histamine crystals suspension caused slight inflammatory reactions. In no case was there a difference between the oil or the oil with histamine, while subcutaneous injection of croton oil, 1:200 in olive oil, caused an exceedingly intense inflammation at the side of injection, very much more violent than that caused by the oil or oil and histamine.

Intramuscular injections of paraffin oil and paraffin oil with histamine crystals in suspension gave no results. The paraffin oil was not absorbed and probably did not permit the histamine to diffuse into the contiguous tissues. In no case was any inflammatory reaction whatever detectable, although the presence of the oil in the muscles was quite obvious.

III. The collodion capsules containing histamine 1:100 in physiological saline called forth marked inflammatory reactions consisting of fibrin, very many polymorphonuclear neutrophilic leucocytes and a few lymphocytes. The inflammatory reaction is most marked around the capsules, which it surrounds entirely, but it can also be traced along the line of separation of the muscles, that is, where the muscles had been separated so that the capsules might be planted. But in no case was the reaction in any way different from that caused by the collodion capsules that contained only physiological saline. Collodion capsules containing 5 per cent lactic acid called forth a very slight reaction, shown by the presence of a few leucocytes and a little fibrin—a picture that contrasted quite strongly with that produced by collodion capsules containing croton oil; in the latter case a violent inflammation was caused. All of the muscles surrounding the capsules were heavily infiltrated with leucocytes; there was most intense reaction around the capsule and all of the blood vessels in the section were greatly distended with reds and leucocytes.

IV. Glass tubes containing histamine do not attract leucocytes *in vivo*.

V. Leucocytes do not wander into glass tubes containing histamine in physiological saline *in vitro*.

CONCLUSIONS

1. A number of experiments were performed to determine the inflammatory inciting and chemotactic properties of histamine.

2. In none of these experiments did histamine produce an inflammatory reaction more marked than that caused by the control experiments.

3. Histamine is indifferent with respect to chemotaxis *in vivo* and *in vitro*.

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TREATMENT OF NON-ENCAPSULATED BRAIN TUMORS BY EXTENSIVE RESECTION OF CONTIGUOUS BRAIN TISSUE*

By WALTER E. DANDY

Gliomata comprise probably 80 per cent of brain tumors; they are never encapsulated, some are circumscribed, others infiltrating. Until just lately, the fact that they invariably recurred has rendered operative procedures apparently hopeless. In recent work, however, we have made trial of careful resection, *en masse*, of a zone of normal brain tissue with the tumor in the center. We have removed an entire right or left frontal lobe without any observable mental or other after-effect. Whole right temporal or right occipital lobes have been removed with only a contra-lateral homonymous hemianopsia. In two instances, it has been necessary to resect the right temporal and the occipital lobe. Practically complete resection of one or two lobes of the cerebral hemispheres has been done in about 10 instances, but partial resection of a lobe with the tumor is frequently all that is necessary. The exact amount of brain tissue which must be removed depends on the position, size and character of the tumor. Partial or even complete resection of lobes of the brain entails very little operative risk. The two largest resections were done when the patients were unconscious, and complete recovery followed.

Similarly, gliomata of the cerebellum can be removed by resecting large portions of the cerebellum. Three times we have removed the entire vermis and no symptoms have followed. Two boys, 10 years old, are well and working, four years after removal of the tumor together with all of the vermis and about half of each lateral lobe of the cerebellum. What parts and how much of the cerebellar lobes can be removed without symptoms, I am not prepared to say. Resections of the cerebellum are more serious than those of the cerebrum,

but in adults there has been a very low mortality.

Whether gliomata of the cerebrum or cerebellum can be permanently cured by this method, time alone must determine. Several of our patients are living and well 3 and 4 years without signs of recurrence.

One cannot always tell from the surface appearance of the tumor, whether a complete resection will be possible. It has never seemed advisable to resect the leg center of the pre-Rolandic area, but in several cases the face area and occasionally the arm center has been removed. Three times it has been necessary to remove Broca's area, apparently completely and to a depth of 2 to 3 cm. Although a complete motor aphasia appeared at once, the speech began to come back in a week or 10 days and gradually returned to normal. In two cases the left occipital lobe has been resected, but not far enough forward to produce visual aphasia. It has never seemed justifiable to attempt to cure a patient of a tumor by resection of the left temporal lobe. In several cases the various ventricles of the brain have been resected. If the dura and scalp are closed very carefully, the open ventricle will do no harm. When tumors in the cerebral hemispheres are localized before paralyses develop, they can be removed without causing paralyses, and if paralyses are present at the time of operation, these will usually become less after the tumor has been removed. The value of early localization of the growth is self-evident.

The removal of these large areas of brain tissue is made possible by the application of Dr. Halsted's well-known principles of careful handling of tissues, and painstaking hæmostasis, so that loss of blood is prevented as far as possible.

II. DIAGNOSIS, LOCALIZATION AND REMOVAL OF TUMORS OF THE THIRD VENTRICLE*

By WALTER E. DANDY

In one patient, a woman, 24 years of age, the only symptoms were those referable to intracranial pressure. By cerebral pneumography, it was determined that each

lateral ventricle was greatly enlarged, but there was no communication between them. Hence we concluded that there must be a tumor in the third ventricle and occluding each foramen of Monro; that the tumor must be small, because neither ventricle was dislocated away from it. A large bone flap was turned down as in a pineal

* Read by title before the Johns Hopkins Hospital Medical Society on April 3, 1922.

approach, and the corpus callosum split posteriorly for about 5 to 6 cm. The right lateral ventricle was then opened through the mesial wall at the septum lucidum. No tumor could be seen; the right foramen of Monro seemed normal on inspection from this lateral ventricle, but a probe would not pass through it, an obstruction

being encountered in the third ventricle. The foramen of Monro was then widened and a small encapsulated, spherical tumor, about 1 cm. in diameter, easily shelled out *in toto*. The tumor was of ependymal origin. The patient recovered. Without cerebral pneumography localization of the tumor and consequently its removal would have been impossible.

III. CEREBRAL VENTRICULOSCOPY*

By WALTER E. DANDY

On two occasions, it has seemed advisable to inspect a lateral ventricle. This was done in one instance through a small cystoscope and in a second an attempt was made with the help of a small operating ventriculoscope to remove and fulgurate the choroid plexus. Both operative attempts were only partially successful, because the instruments, in their relatively primitive construction, were not quite adaptable, and it therefore became necessary to remove the plexus in the usual way. The inspection of the ventricles was all that could have been desired. It was possible to see practically the entire extent of the lateral ventricle, the foramen of Monro, the septum lucidum with numerous perforations in it, and the entire extent of the choroid plexus including the

glomus. The blood vessels in the wall of the ventricle were easily visible. In one instance, we could distinguish the opening of a defect in the wall of the ventricle—the mouth of an encephalocele. The indirect means of determining the intracranial contents are now so satisfactory that ventriculoscopy will be indicated only occasionally. There may, however, at times be cases in which a direct inspection will be useful. How useful the operating ventriculoscope will be, can scarcely be predicted. I have used both air and water as media in the ventricles; the former, which can be easily introduced, is by far the better. For inspection alone, the instrument introduced into the ventricle need be little larger than the ordinary ventricular needle.

IV. AN OPERATION FOR THE REMOVAL OF LARGE PITUITARY TUMORS*

By WALTER E. DANDY

This procedure is an extension of Heuer's operation, which is by far the most practical and useful of all yet suggested. In many cases, when the tumor is small or when the lesion is a simple cyst, Heuer's approach is adequate, but most pituitary tumors are very large when they come for operation, so that total removal is possible only with much more room. The exposure of the tumor is enlarged 4 or 5 times by section of one optic nerve. The nerve supplying the eye in which the vision is worse, or in which the vision has been first lost, is chosen for section. If the difference is slight, the right eye is always selected.** This supplementary procedure renders it possible to retract the brain to such an extent that

almost any tumor of the pituitary can be completely removed. Adenomas of the pituitary (the most frequent hypophyseal tumors) are first resected intracapsularly, the loose capsule is then pulled forward in stages and eventually removed flush with the sella. This method I have used for the past four years. Up to the present time we have not had a recurrence, two patients having gone 4 years. The vision in the intact eye returns, the exact extent of recovery depending on the duration of blindness before operation. Complete removal of these tumors is, of course, a serious operation, the danger, in a general way, being in proportion to the size and character of the tumor and the strength of its capsule.

V. AN OPERATIVE PROCEDURE FOR HYDROCEPHALUS*

By WALTER E. DANDY

Strictures of the aqueduct of Sylvius recur after any attempt to restore the lumen. For this reason, if treat-

ment is to be successful, the fluid must be sidetracked into its normal channels. With this in mind, a procedure which apparently is anatomically correct has been devised, to supersede any direct attack on the aqueduct. This consists in removing the floor of the third ventricle. A small opening is made in the skull and dura in the frontal region, the frontal lobe is elevated until the

* Read by title before the Johns Hopkins Hospital Medical Society on April 3, 1922.

** These patients are nearly always blind in one or both eyes at the time of operation.

bulging third ventricle is well exposed. Usually, it is necessary to divide one of the optic nerves, for in hydrocephalus these nerves are very short; usually the chiasm lies directly on the anterior border of the sella turcica. This opening in the floor of the third ventricle affords an exit from the dilated ventricles, so that the fluid can now pass directly into the cisterna chiasmatis and interpeduncularis—the normal distributing centers for cerebrospinal fluid. We now have proof that an opening made in the fourth ventricle will remain permanently patent, and this encourages the hope and belief that this opening in the floor of the third ventricle will also be permanent. The ventricular wall is a very thin membrane and offers a minimum of glia tissue to repair the defect.

This procedure is by no means analogous to making an opening in the roof of the third ventricle. The latter can have no beneficial result because the fluid escapes into the subdural space where the absorption is slightly, if at all, greater than in the ventricles. Moreover, the opening is through cerebral tissue which proliferates and closes it, unless a good deal of the brain has been destroyed.

We have employed this method 6 times. No claim is made for its success. Time alone will decide. If successful, it should also be applicable to occlusions of the foramina of Luschka and Magendie and to those cases of communicating hydrocephalus in which the cisterna chiasmatis and interpeduncularis are patent.

VI. DIAGNOSIS AND LOCALIZATION OF SPINAL CORD TUMORS*

By WALTER E. DANDY

In 1919, a report of intraspinal injections of air was presented. In several cases, the air was found in skiagrams of the head and proved to be useful in defining blocks in the subarachnoid space, resulting from tumors or inflammations. The air was shown in the upper part of the spinal canal and it was suggested that blocks of the spinal canal should be similarly demonstrable or could be excluded when not present. In five cases of possible spinal cord tumors we have made intraspinal injections of air. In four, it passed through into the cranial chamber and was demonstrable in the subarachnoid space and the ventricles, so that tumors could be excluded, and other diagnoses could be considered.

In the case of one of Dr. H. Thomas's patients the upper margin of the air was shown at the fifth cervical vertebra; sharp pains were at once carried down the sixth cervical nerves on each side (the site of the tumor). No headache

occurred in this case, because the air did not reach the cranial chamber. The skiagram of the head also failed to demonstrate air either in the cerebral sulci or in the ventricles. As the Wassermann for the blood and spinal fluid was positive, antisyphilitic treatment was instituted with the result that the patient recovered completely from the paralysis. Before his discharge from the hospital, the intraspinal air injection was repeated; headache at once appeared; and air was shown in the skiagram of the cerebral ventricles. No pains developed along the cranial nerves at this time. It was evident that the tumor had been absorbed, and that air could pass freely through the region which previously had been blocked. This method should give the same results as the combined spinal and cisternal punctures of Ayer. It is hoped that the air shadow will be sufficiently clear not only to permit the diagnosis of a subarachnoid block but also to establish its localization. How sharply shadows in the thorax and abdomen will be defined, I do not know.

* Read by title before the Johns Hopkins Hospital Medical Society on April 3, 1922.

SPONTANEOUS LABOR OCCURRING THROUGH AN OBLIQUELY CONTRACTED, KYPHOTIC, FUNNEL PELVIS*

By J. WHITRIDGE WILLIAMS.

This case seems worthy of being recorded for two reasons—first, because it affords a striking illustration of the mechanism by which certain skeletal changes may affect the shape of the pelvis, and secondly, because the conservatism with which the labor was conducted afforded conclusive evidence of the ability of the uterine cicatrix following Caesarean section to withstand the strain

of labor, as well as to demonstrate the fallacy of the dictum "once a Caesarean, always a Caesarean."

The patient was a twenty-one year old colored girl, who was markedly deformed by kyphotic changes in the vertebral column and by ankylosis of the left hip and knee originating from tuberculosis developing during the second year of life. The two previous pregnancies had been ended by Caesarean section in another city in 1919 and 1920 respectively, and she was admitted to the ward on September 26, 1921, under the belief that she was in

* Case report made before the Johns Hopkins Medical Society, Dec. 5, 1921.

labor at term—the last menstrual period having begun on December 20, 1920.

In view of the previous history, the woman entered the service for a third Caesarean section and, as she was anxious to avoid the possibility of its repetition, it was tentatively proposed to remove the uterus by supra-vaginal amputation after evacuating its contents. On examination, there were occasional uterine contractions, and a small child presented in R. O. P. with its head deeply engaged in the pelvic cavity. In the absence of disproportion, I declined to do a radical operation and predicted a spontaneous outcome, but advised that forceps be applied early in the second stage in order to prevent unnecessary strain upon the scars of the previous sections.

As it proved that the patient was not in labor, I examined her thoroughly the next day with the following results: a frail young woman weighing 83½ pounds and measuring 130 cm. was lying on her back in bed. The heart and lungs were normal. The thorax was unusually arched and, with the large sagging breasts and pendulous abdomen, presented a peculiar picture. The fundus of the uterus reached to within three fingers of the xiphoid, and the abdominal enlargement projected in such a way that its lower part extended vertically downward. A jagged Caesarean section scar extended one-third above and two-thirds below the umbilicus. A small child lay in R. O. P. with the head deeply engaged. In the left groin just below Poupart's ligament was an old scar indicating the point of discharge of a psoas abscess. The left leg was atrophic as compared with the right, and was fixed at the hip-joint in slight flexion. The knee was flexed and completely ankylosed, while a long indrawn scar upon its posterior surface indicated the site of an operation which had been performed when the patient was five years of age.

Upon inspecting the standing patient from the front, (Fig.1) one was impressed by the extreme shortness of the torso, the pendulous condition of the abdomen, and the fact that the body weight was supported entirely by the right leg, while the toes of the left foot scarcely touched the floor. The abdomen was so pendulous that no trace of the abdominal cicatrix was visible, and the umbilicus lay 7 cm. below a line joining the iliac crests. When viewed from the rear, four striking features were noted. First, the presence of a double kyphosis, with one gibbus in the upper dorsal and the other in the lower lumbar region; second, tilting of the entire vertebral column so that the right shoulder was higher than the left; third, collapse of the torso to such an extent that the lowermost ribs were in contact with the iliac crests, which was indicated externally by a deep transverse furrow of the skin on either side; and fourth, oblique tilting of the pelvis so that the right iliac crest was 2 cm. higher than the left.

As is shown by Figure 2, there is nothing remarkable about the upper gibbus, but the lower one, which is separated from the former by a moderate lordosis, is of interest, partly on account of its location, but particularly because its lower limb passes directly into the sacrum. The existence since childhood of such a lumbar kyphosis led us to suppose that the pelvis would be funnel-shaped, while the unilateral lameness would give rise to an oblique contraction. Careful examination confirmed both suppositions.

External pelvimetry gave the following measurements: 21, 23, 27 and 17.5 cm., and showed that the pubic arch was asymmetrical and somewhat narrowed, with the transverse and anteroposterior diameters of the outlet measuring 7.25 and 10.75 cm., respectively. On internal pelvimetry, the diagonal conjugate was found to measure 12 cm. and the entire right side of the pelvis was flattened and pushed upward, inward and backward, while the left side presented normal curvatures. At first glance it appeared that we had to deal with a typical generally contracted funnel pelvis, associated with oblique contraction resulting from the unilateral lameness. Closer consideration showed that this was not the case, as the diagonal conjugate of 12 cm., although slightly shorter than normal, was disproportionately large for such a pelvis. On the contrary, we were forced to conclude that it was really relatively lengthened, while both the transverse and anteroposterior diameters of the inferior strait were absolutely shortened. In other words, all of the criteria for a kyphotic funnel pelvis were fulfilled.

Figure 3, which represents an X-ray of the superior strait, clearly shows the existence of the oblique contraction and demonstrates that it had resulted from the unilateral lameness. In this instance, the patient since early childhood had supported her body weight almost exclusively upon her right leg, with the result that with each step a greater upward and inward force had been exerted through the right than through the left acetabulum, and consequently the right side of the pelvis had become flattened and slowly forced upward, inward and backward, while the left side had developed normally, thus giving rise to an oblique contraction involving the entire pelvis, but more particularly the superior strait, which has assumed an obliquely ovate form. That the contraction was not more pronounced is probably attributable to the fact that the patient has always walked with a crutch, and thereby diminished to some extent the extreme mechanical possibilities of her lameness.

Reverting to the kyphosis. The presence of a "hump back," no matter what the situation of the gibbus, is always a source of anxiety to a pregnant woman and causes her to anticipate serious difficulty at the time of labor. Experience, however, shows that such fears are generally exaggerated, as the great majority of kyphotic deformities do not lead to serious dystocia. This is due

to the fact that when the gibbus is situated in the cervical or dorsal region, or even when it is dorso-lumbar in character, the development of a marked lordosis below it results in such compensation, that the body weight is transmitted to the base of the sacrum in such a manner that the pelvis is not affected. On the other hand, as has been shown by the researches of Breisky, Chantreuil, Tarnier and others, when the kyphosis involves the lumbar region, there is no longer any possibility for compensation, as the lower limb of the gibbus articulates directly with the base of the sacrum, with the result that the body weight is transmitted to the upper end of the lower limb of the gibbus in such a way that a parallelogram of forces is developed which resolves itself into two factors—one directed downward and the other downward and backward. The latter tends to displace the lower limb downward, and at the same time to draw it backward. As its lowermost extremity is firmly united to the base of the sacrum, this results in a rotation of the sacrum about its transverse axis so that its base becomes retropulsed, while its tip is displaced forwards, thereby increasing the length of the conjugata vera and diminishing that of the antero-posterior diameter of the inferior strait. At the same time the sacro-iliac joints are spread apart, with the result that the innominate bones rotate about their transverse axes, so that the ischial spines and the tubera ischii approach one another and thus diminish the transverse diameters of the plane of least pelvic dimensions and of the inferior strait with the production of a funnel pelvis, as is demonstrated by the example before us.

In most kyphotic funnel pelves which have been studied in the dry state a further change has been noted—namely, a lengthening of the sacrum from tip to base, as well as a slight diminution in its concavity arising from the fact that the traction exerted upon its base tends to draw the bodies of the sacral vertebrae out beyond the level of the alae. Whether this was present in the pelvis under discussion I am unable to state, but no evidence of it was apparent upon palpating the sacrum or in the X-ray plate.

The patient fell into labor early in the morning of October 13, 1921, and three hours and twenty minutes later vaginal examination showed that the cervix was fully dilated and the head on the pelvic floor in R. O. A. The membranes ruptured spontaneously at this time, and low forceps were applied in order to spare the uterine cicatrix the strain incident to expulsion, and a male child weighing 2420 grams and measuring 46 cm. in length, with a biparietal diameter of 9 cm., was easily delivered without injury to the perineum.

The convalescence was uneventful except for a mild colon bacillus pyelitis, which developed three days before the onset of labor and caused a febrile reaction, which continued until the fifth day of the puerperium. The

patient was discharged in excellent condition on the 15th day and suckled her child, which on discharge exceeded its birth weight by 80 grams.

The labor was of interest from two points of view. First, that the small child passed through the abnormal pelvis without difficulty, and secondly, that neither the distention of the uterus incident to pregnancy nor the strain of the second stage of labor had any untoward effect upon the scars of the previous sections.

Through the courtesy of the hospital at which the previous Caesarean sections had been done, we learned that the children weighed 2880 and 2490 grams, respectively, as compared with 2420 grams in the present instance. This at once raises the question as to whether radical operative interference was necessary. While criticism is not permissible unless one is in possession of all the facts, it appears probable that the second labor, at least, would in all probability have ended spontaneously had it been given a chance, and that the actual indication for the second Caesarean section was fear of the behavior of the uterine scar following the first operation. The result in our hands shows that such fears were groundless, and brings us to the discussion of the behavior of the Caesarean scar in general.

A priori, one would be inclined to believe that the scar tissue in the cicatrix would represent a *locus minoris resistentiae*, which might predispose to rupture in subsequent pregnancies, and the actual occurrence of such accidents indicates that the danger is a real one. I shall not, however, discuss the question at any length, as an extensive article by Dr. Thomas O. Gamble based upon the experience of our service has just been published.

In Gamble's paper, evidence was adduced to prove that, while rupture sometimes occurs, its frequency is much less than is currently taught. This is due to the fact, which we first demonstrated, that when the uterine wound has been properly sutured, and infection has not occurred, microscopic examination shows a surprising lack of scar tissue, and demonstrates that muscular fibres cross the site of the old incision in all directions just as if it had never been made. Indeed, in a number of uteri which were removed at a second or third section, no trace of the previous incision could be discovered, except perhaps a slight furrow upon the external and internal surfaces of the anterior uterine wall, and in certain specimens even this was lacking.

On the other hand, if the incision has not been properly sutured, and more particularly if infection has occurred, good union may not obtain, with the result that the uterine wall is thinner at the site of the old incision than elsewhere. In such circumstances, it is conceivable that, as the distention of the uterus increases, the thinning may become so accentuated as to lead to rupture with its serious consequences.



FIG. 1.—Showing shortened torso, pendulous abdomen and body weight borne upon right leg.



FIG. 2.—Showing double gibbus and collapse of torso.



FIG. 3.—Showing obliquely ovate superior strait.

The case before us offers additional proof that the uterine scar can withstand the strain of subsequent pregnancy and labor, and again demonstrates that the dictum "once a Caesarean always a Caesarean" does not always hold good. Indeed, my experience leads me to believe that its importance has been greatly exaggerated. At the same time, it should always be remembered that,

as the Caesarean scar may represent a *locus minoris resistentiae*, this fact affords the best possible argument for restricting the employment of the operation within the narrowest limits, instead of employing it more indiscriminately as is advocated by so many obstetricians and surgeons.

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THE EVOLUTION OF HUMAN RACES IN THE LIGHT OF THE HORMONE THEORY*

RACIAL STATUS AND FORM OF BODY

By Prof. SIR ARTHUR KEITH

(Conservator of the Museum and Hunterian Professor, Royal College of Surgeons, London, Eng.)

HERTER LECTURE No. 2.

Giantism and Acromegaly.—Giants are usually the subjects of acromegaly as well as of overgrowth of trunk and limbs. There have been authentic cases of giantism which were free from the usual manifestations of acromegaly. There are also cases, certainly few in number, of adolescents who were the subjects of acromegaly and yet showed no increase in stature. From such instances one infers that, although giantism and acromegaly may be present in the same subjects, they represent disturbances of two correlated yet distinct growth mechanisms. During the years of growth there must be some ever-

sent process at work controlling the growth of the correlated functional systems of the body. Since the pituitary is enlarged in giants, who are free from acromegalic traits, we may infer that this mechanism is also represented in the pituitary—in part at least—for other centres of growth also influence stature. Giantism represents a disorder of the hormone mechanism presiding over, not the reactional, but the normal growth of parts.

Localized Acromegalias.—In seeking to unravel the nature of acromegaly one is at first tempted to suppose that the condition is merely the result of the pituitary throwing increased doses of a growth-elixir or hormone, into the circulatory blood. A study of cases of localized overgrowth or partial acromegaly shows that the expla-

* Abstract of Lectures Nos. 2 and 3 of the thirteenth course of lectures under the Herter Foundation, delivered before the Johns Hopkins University, October 6, 1921.

nation cannot be so simple. The most instructive cases of this kind are those seen in children in whom there is a marked unilateral hypertrophy of the apparatus of mastication. One side of the face is acromegalic, the other is normal. A minute examination of such cases shows that the overgrowth is sharply limited to the parts concerned in mastication—to one half of the mandible, to the corresponding maxilla and palate, and to the bones which give attachment to the corresponding muscles of mastication. The tympanic plate is involved in the overgrowth, showing that it is part of the apparatus of mastication. The disorder must commence in foetal life, for the teeth of the affected side are much larger than on the normal side. There is no evidence as yet that there is an anatomical change in the pituitary glands in such cases, but as we have here an undoubted manifestation of localized acromegaly, it is legitimate to infer that we must be dealing with a disturbance of the same hormone-machinery that lies behind generalized acromegaly. In such cases we see the anatomical elements of a single functional unit picked out. In generalized acromegaly all functional units are affected.

The mechanism of Growth Hormones.—These localized cases of acromegaly show that growth is not merely the result of the presence of an activating substance thrown into the general circulation by one or by a number of glands of internal secretion. A local as well as a general mechanism must be concerned. In the case of *functional* hormones, such as secretin and adrenalin, and also in the case of *growth* hormones formed within the testicle, we see that such substances, although offered to all the tissues of the body are selected and accepted only at certain definite points or regions. It is not difficult to conceive that one of these local receptive mechanisms might be heightened in avidity or susceptibility and thus give rise to a local hypertrophy. Whatever the nature of the central and peripheral growth mechanisms may prove to be, there can be no doubt that they are arranged on a physiological basis—one which bends a collection of anatomical elements to serve definite functional ends. It is this complex dual mechanism which is inherited from generation to generation and which fashions mankind into different functional types and races.

Examples of the Pituitary Mechanism at Work in the Evolution of Types.—Up to this point I have endeavoured through a study of acromegaly to unravel the complex mechanism which regulates the growth of the human body, concentrating attention more especially upon the apparatus of mastication. To exemplify the manner in which the pituitary growth mechanism produces its effects, no finer example can be chosen than man's nearest relative—the gorilla. He is the greatest and strongest member of the zoological group to which man also belongs—the Higher Primates. The skull of the baby gorilla is smooth and rounded like that of a child; after

infancy there sets in a series of growth changes which transform the whole appearance of the individual; the masticatory apparatus reaches a supreme degree of development; the temporal lines rise up to form the great sagittal and lambdoidal crests of bone, the lower part of the frontal bone is extended forwards until it forms a great ledge-like bar of bone above the orbits; the jaws and face become massive. Behind this transformation must lie the same pituitary growth mechanism which is revealed in the acromegalic. In the chimpanzee the growth machinery comes to a standstill at a stage reached in the juvenile gorilla; in man it is arrested at a stage represented in the childhood of the gorilla. In Neanderthal man we also see the pituitary mechanism vigorously at work. The ape-like supraorbital ridge, the massive jaws, and the wide expanse of the nuchal platform are evidences of this influence. But it is among peoples of the Caucasian or European type that we may best mark the peculiar effects of this mechanism among modern races of mankind. The homeland of this type extends from the western confines of India to the Atlantic shores of Europe. So far as the facial features are concerned, a long narrow prominent nose, a prominent chin with a tendency to elongation and narrowing of the face are the chief racial marks of this type. These are features which almost invariably become greatly exaggerated in the acromegalic. In the shaping of the European type, the pituitary mechanism has had much to do. In shaping the racial characters of the European type the pituitary influence seems to have dominated the other centres of hormone-production; in the Mongolian and Negro types other centres, such as the thyroid and adrenals seem to have gained in potency.

The Origin of new racial characters and types.—Although I have by no means exhausted the anthropological lessons to be derived from the study of pituitary disorders—such as are seen in giantism, Fröhlich's disease (dystrophia adiposo-genitalis), dwarfism, infantilism, progeria—it is important, before we proceed further, to study the manner in which new racial characters come into existence—particularly those relating to the colour of skin and hair and to the nakedness of the human body. In the Museum of the Royal College of Surgeons there has been exhibited since the conservatorship of Sir Richard Owen, the pregnant uterus of a chimpanzee, dissected so as to expose a fetus in the seventh month of development. Prof. Louis Bolk of Amsterdam has a similar specimen. Independently he and I have realized the important bearing which such specimens have on the theory of hormone evolution.* So human are the uterus and fetus in appearance that a superficial observer might well believe he had before him the womb and fruit of a woman. The skin of the body is nude, except for the

* "The Part Played by the Endocrine Glands in the Evolution of Man." *Lancet*, 1921, XI, 588.

presence of a fine lanugo; on the other hand, the scalp is covered with hair of a brown colour. The distribution of hair is human—not anthropoid. The skin is scarcely pigmented; it is ashen grey in colour; at a corresponding stage the negro foetus is not dark skinned. Prof. Bolk draws the conclusion, and I agree with him, that man has come by his naked skin and the European by his white colour by inheriting a foetal condition from his anthropoid ancestry. A transient foetal condition of the anthropoid has become fixed permanently in the human adult. We have seen that in his masticatory apparatus and in his nuchal fixation, man retains stages seen in the childhood of anthropoid apes. Man's new characters have first been elaborated within the uterus of the ape; later these foetal characters have been incorporated in the make-up of the human adult.

Up to this point Prof. Bolk and I proceed together. But we must go further and ask ourselves the question, How do these foetal characters arise in the anthropoid womb? I have laid stress upon the fact that the pituitary mechanism—the whole mechanism of growth hormones—has been elaborated and organized on a functional or physiological basis. This was made apparent when dealing with the effects of the pituitary mechanism in fashioning the apparatus of mastication. Nor do we make any extravagant demands on our imagination when we proceed to suppose that such hormone mechanisms are constituted and elaborated during the foetal life, in the same way as the mechanisms of instinct and of reflex action are given a concrete representation in the nervous system. The nerve-machinery of an automatic action is inherited. One may hazard the presumption that this also may be true of the hormone machinery, which presides over the functional fashioning of the body. The long developmental intra-uterine period of higher vertebrates, particularly of the higher primates, gives the hormone system the shelter necessary for working out its effects in the foetal body and the opportunity of attempting fresh experiments. It is under the influence of this automatic hormone mechanism that new characters are elaborated and fresh experiments launched during the developmental stages of life. Darwin's Law of Natural Selection tests these experiments, accepting the successes, and rejecting the failures.

Hair and Skin Characters.—Colour is one of the principal marks of race, but in explaining why we should find all shades from the deep black of the negro to the fair skin of the Norseman, we are handicapped by our ignorance of the part played by pigment in the economy of the body. The facts at our disposal are these: When the medullary parts of the adrenal bodies are injured or destroyed by disease, the skin becomes pigmented—as Thomas Addison noted in 1855. John Hunter inferred that the original colour of man's skin was black and all the facts we have gathered since his time favour this

view. The gorilla is the negro amongst anthropoids, he is deeply pigmented at birth. The chimpanzee darkens after birth, some races early, others later. In the orang the hair is red but the skin has a slaty hue. We have already seen that the foetus of man, as of anthropoid, is free from pigment.

There is thus a relationship between pigmentation and these important centres of hormone production—the adrenal glands. We obtain some light on our problem when we remember that the adrenals are closely connected with the important function of maintaining the body at a temperature—under all extremes of heat or cold—of approximately 98.4° Fahr. The regulation of the blood supply to the skin, the secretion of sweat, and the development of subcutaneous fat, are the important means by which this end is secured. The development of hair on the body, the amount and distribution of pigment and fat must also be accessory circumstances in the regulation of temperature. We have seen how anatomical elements are combined into functional units by means of a hormone mechanism and I infer that future research will reveal a growth mechanism which presides over the parts concerned in the regulation of temperature and that skin-pigment will be found to be included in this system. The mechanism must be a complex one in which pituitary and thyroid are also involved, for there is no doubt that disorders of the pituitary are followed by definite changes in hair and skin; thyroid disorders also give rise to changes, but they are of another kind. From these facts we see that the skin is dominated by a hormone mechanism in which adrenal, thyroid and pituitary are combined—a mechanism of a complex nature.

Thyroid Effects.—On entering into a discussion on the effects produced by thyroid substances in shaping the racial characteristics of mankind, it is necessary to recall some of the statements made regarding the nature of the machinery lying behind the growth changes produced by hormones. I am regarding the glands of internal secretion as centres for the elaboration and distribution of hormones and believe that the organs and tissues of the body are endowed with special affinities for the hormones in circulation and that very probably these local centres have also a means of making their needs known at the centres of hormone production. In dealing with pituitary effects I have proceeded on the belief that we are witnessing positive results—an increased action of the pituitary producing increased activity in definite peripheral sites of growth. Thyroid substances can produce acceleration of growth; this is strikingly shown by the manner in which extracts of the gland can increase the growth of cretinoid children and hasten the metamorphosis of tadpoles. I presume that thyroid and pituitary growth hormones utilize the same local or peripheral mechanism in producing their effects.

Cretinism.—It is, however, not the positive or exaggerated action of the thyroid, but its minus or defective one which brings out the part played by this gland in the differentiation of races. In cretins, growth of all the tissues of the body is not only retarded but positively distorted. The effects are best seen in the cartilaginous growth discs of long bones. The cartilage cells multiply slowly and irregularly; osteoblasts cease to have the power to invade and overcome them. The cartilaginous base of the skull suffers in this way; the root of the nose becomes drawn in and widened; the nose itself, which is built over a foundation of cartilage, is wide, short and not prominent. The face, which in acromegaly is long and narrow and wedge-shaped, is short, wide and flat in the cretin. The skin is dry, the hair scanty, and the subcutaneous tissue thickened. These are the results of a gross deficiency of thyroid substances. When thyroid extracts are administered, the characteristics previously produced disappear and are replaced by the normal. We have in cretinism positive evidence that the thyroid has to do with regulation of stature and the determination of characters of face, hair and skin—all being marks employed by anthropologists in the classification of races.

Mongolism and Achondroplasia.—Cretinism is a result of a gross deficiency of thyroid substances. There are at least two disorders of growth—mongolism and achondroplasia—which one has reason to attribute to a defective or altered action of thyroid substances. It is true that the exhibition of ordinary thyroid extracts has no effect upon such cases, nor has any one succeeded in producing these conditions experimentally in animals, yet there are clinical manifestations and structural alterations in both of these disorders, which are also seen in cretinism, and for this reason I feel justified in attributing both mongolism and achondroplasia to disturbances in the complex effect exerted by thyroid substance on the growth and differentiation of the body. Dr. Langdon Down, in 1866, recognized that a certain class of imbecile was characterized by traits which recalled those seen in the faces of Mongolian peoples. My friend, Dr. Francis Crookshank has explained the appearance of such traits as a harkback to a Mongolian ancestry. Not only is there a lack of evidence that a Mongolian people has at any time populated Europe, but there is circumstantial evidence that the Mongolian type of humanity is one of the most recently evolved of racial forms. The theory which best explains all the facts is to suppose that Mongolian features arise under a peculiar or altered action of the growth mechanisms centered in the thyroid gland.

It is not necessary to recall the bodily characteristics of achondroplasia or "bulldogism"—for the disturbance of growth is that seen in all varieties of the bulldog breed. The trunk is normal in size but all segments of the limbs, including shoulder and pelvic girdles, are retarded and distorted in growth. In particular, the base

of the skull suffers;* the cerebellar fossa and the area of nuchal fixation are reduced in size and greatly altered in shape; the root of the nose becomes drawn in between the eyes, and broadened just as in cretins. The facial bones laid down over the nasal capsule are retarded in growth, so that the anterior part of the maxilla is drawn up, producing a pug-face. As in dachshunds, the limbs may be affected while the base of the skull and nose develop normally. The disorder occurs in all races of mankind and in varying degrees of severity. Darwin described a bulldog breed of cattle from the Argentine. Dr. Seligman found that the cretinoid or bulldog calves of Kerry cows had disordered thyroids. Dr. Douglas Symmers observed that in a proportion of still-born achondroplastic children there was a structural disorder of the thyroid gland. In achondroplasia we are clearly dealing with a disorder of a growth mechanism which is widely distributed in the animal kingdom and the evidence points to this mechanism having its centre in the thyroid gland.

Now Mongolian peoples are characterized by limbs which are short in comparison with their trunk length. The nasal region between the eyes is wide, flat and frequently sunken in typical Mongols. The flattening of the root of the nose is even better seen in the true Negro type. In the Mongol, as in the cretin, eyebrow ridges are poorly developed or absent. It is not only amongst human races that these supposed thyroid effects can be traced. The orang is the bulldog or Mongol amongst anthropoid apes; he is pug-faced, the nasal region of his face being greatly reduced and drawn in, the supra-orbital ridges are developed to only a minor extent—especially when contrasted with their development in the gorilla. With this conformation goes a silent and rather morose temperament. The orang differs in colour from other anthropoid apes just as the Mongolian colouring contrasts with that seen in the negro or European. The late Prof. H. Klaatsch was struck by the points of resemblance which linked the Mongolian and orang types and sought to explain the similarity by supposing that the Mongol and orang had a common ancestry. The explanation I offer is that both have inherited a common mechanism of growth-hormones and they are superficially alike because, in both, the thyroid effects of this mechanism have become dominant.

Dwarf-Races and Stature.—No anthropological problem has been more discussed than the position to be assigned to pygmy or dwarf varieties in the scale of human evolution. In finding the proper solution to this problem, it has to be remembered that all living dwarf breeds are members of the negro race and that each dwarf variety has many resemblances to the breed of negro existing in the same region. The central African pygmies are dwarf

* Quart. Journ. Med., 1912, Vol. 5, p.157.

forms of the true negro; the Bushman is a dwarf form of the Hottentot breed and the pygmies of the Far East are varieties of the Eastern Negroids. From their characters and distribution one infers that pygmy peoples have been produced locally from normal members of the negro race, just as dwarf races of horses, dogs and cattle have arisen locally from breeds of normal size. It is also worthy of note that the Dinkas and Shilluks of the White Nile are amongst the slenderest and tallest examples of mankind. Stature is a highly variable factor in the negro race. When we attempt to disentangle the exact nature of the growth mechanism which leads to the production of dwarf races, we become keenly alive to the complexity of the problem and to a need for further knowledge. All tissues of the dwarf body are fashioned on a miniature scale, but we can best realize what happens by concentrating our attention on the epiphyseal lines or growth discs of long bones which are so directly related to stature. Epiphyseal lines represent local growth mechanisms, activated and controlled by substances thrown into the circulation at centres of hormone production. Pituitary substances play on these sites of growth; we explain giantism by an oversupply, and infantilism, such as is seen when the pituitary is compressed by a cyst, by an under-supply. The sexual glands can also influence the rate of growth at epiphyseal lines, but they exert their influence not directly on the cells of the epiphyseal lines but indirectly through the cortical part of the adrenals. A fuller knowledge of the part played by the growth hormones of the thyroid is likely to give us an explanation of the origin of dwarf breeds.

The Hottentot Type.—The wide, flat interocular field of the typical Mongol is to be regarded, so I think, as an effect of thyroid action; the same inference must also hold good in the case of the Negro. Now, in the most peculiar breed of existing Negroes—the Hottentots of South Africa—the interocular field is particularly wide and flat; the bony skeleton of the nose is reduced; the supra-orbital ridges are poorly developed. Many anthropologists have noted Mongolian traits in the Hottentot face, as well as the yellowish tint of their skin. These traits are also to be noted in the face and skin of the Bushman. If we admit that there is a tendency for the thyroid mechanism to become dominant in the Negro type, then we can explain the appearance of Mongolian traits in the Hottentot—undoubtedly a member of the Negro race. No other theory can give a satisfactory explanation of the racial features and of the distribution of the varieties of the Negro-type.

Dystrophia adiposo-genitalis.—Having touched on the Mongolian traits of the Hottentot, it is convenient at this point to discuss another anthropological character by which the females of this racial type are marked—a tendency to the accumulation of fat on the buttocks and outer aspect of the thighs. This peculiar character occurred in certain peoples who lived in Europe towards the close of

the ice age, as we know from discoveries of statuettes and drawings of that distant period. These fat-buttocked women were of the European type, although their skulls do show some negroid traits. The changes which occur in modern women at the menopause help to throw some light on the physiological machinery underlying the appearance of such a feature as a race mark, for, as is well known, atrophy of the ovaries is frequently followed by the deposition of fat, particularly in the region below the waist. The same result frequently follows removal of the ovaries or testes. In the growth disorder first described by Fröhlich—*dystrophia adiposo-genitalis*—there is a like tendency to the subcutaneous accumulation of fat, but in such instances deposition follows enlargement of the pituitary gland and an atrophy of the sexual system. On our present evidence, we cannot say whether the change in the pituitary or in the sex glands is the primary one—very probably it will prove to be the latter—but we may safely attribute the deposition of fat and the assumption of a female configuration of body as a direct result of the sexual atrophy. In Hottentot women the physiological process underlying the localized deposition of fat comes into play independently of any loss of sexual function and yet upon the evidence of pathology we must suppose that this process is controlled through a hormone mechanism centred in the sex glands. There is ample evidence to show that a close functional relationship exists between the centres of hormone production—the adrenals, pituitary, pineal and thyroid glands. The sex glands have apparently the power, by means of ovarian or testicular hormones, to influence and set into operation the growth-controlling machinery situated in the glands of internal secretion. All other evidence points to the influence of the sex glands as being one which is exerted, not directly on the tissues of the body, but through the intermediation of other centres of hormone production. The deposition and absorption of fat we must regard as controlled by an interaction between adrenals and thyroid—the glands concerned in regulating the metabolic rate and temperature of the body. The regional deposition of fat recalls, as do localized acromegalias or overgrowths, the need for postulating a local as well as a central machinery in our explanation of the efforts of growth-hormones. It is worthy of remark that the profuse wrinkling of the face, so commonly seen in adult Bushmen and Hottentots, is also present in eunuchs and eunuchoid beings. In these conditions the texture and elasticity of the skin are also affected.

The Nilotic Type of Negro.—Before leaving the Negro type, from which I have selected the foregoing instances to illustrate the application of the hormone theory to the explanation of racial features, there is a further example I may be allowed to cite. The Negro tribes along the White Nile are slender and tall, their height being largely due to length of limb. Their extremities, in opposition to

those of the Mongolian type, are long in comparison to length of trunk: Now castration, as is well known, results in a delayed closure of the epiphyseal lines. Eunuchs are taller and longer limbed than are normal men belonging to the same place and race. At puberty, when the influence of the sex glands begins to play on the other hormone centres, the body loses the slenderness of boyhood and girlhood; in the Nilotic negro type the slenderness of youth is retained in adults—a further example of the evolution of a new racial type by the fixation of parts at an immature phase. The sex glands in this type appear to reach maturity without setting in motion the machinery which expands the framework of the body and thus undoes the slenderness of youth. It is an example similar to that cited in the Hottentot women—where menopause characters appeared without impairment of the sexual function of the ovaries.

HERTER LECTURE NO. 3.

Sex-Glands and Adrenals.—From remote times mankind has known that removal of the sex glands completely alters the characters of body and mind of the human as well as of the animal body. What is new in this department of knowledge is the hormone theory—an explanation of how the effects of castration are brought about. Until the postulation of the theory of hormones by Starling in 1904, medical men had supposed that the parts and systems of the body were provided with only one means of intercommunication—a nervous or telegraphic system. By 1904 knowledge had reached a point where it could be seen that there was an infinitely older system of intercommunication—a postal or hormonal system—which as regards the growth of the body is very much more important than the nervous system.

Tumours of the Adrenal Cortex.—The important rôle of the pituitary gland as a regulator of growth was an accidental discovery. Clinicians and pathologists discovered that disorderly overgrowth of the body was accompanied by an enlarged and adenomatous condition of this gland. Medical science benefitted by Nature's pathological experiment. In exactly the same way we have learned that the cortex of the adrenals is an important centre of

growth-control. This glandular tissue is also liable to become the site of a localized overgrowth or an adenoma in the young and is accompanied by a very definite series of growth changes in the body. Recently my friend Prof. E. E. Glyn* of Liverpool has rendered an important service by tabulating the data relating to cases of tumour of the adrenal cortex, adding several instances which have come under his own observation. I had an opportunity of seeing the classical case described by Bulloch and Sequeira in 1903. The occurrence of such tumours in young boys is accompanied by a premature development of all the bodily changes which should not take place until the age of puberty. A boy of five years of age will become sturdy and thick-set—an infant Hercules; his voice will break; hair will grow on the face and pubes; his penis becomes developed; the testes become active. The adrenal cortex presides over the development of all of the structural parts which are fashioned into "secondary sexual characters"—the name given to them by John Hunter. Cases are recorded in which these changes have set in soon after birth—pubertal changes masking the features of babyhood. In female children or in young women, cortical tumours of the adrenal produce a different effect; they tend to bring out the sexual characters of the male. With the surgical removal of such a tumour, the normal female configuration of body, voice and of mind becomes restored, the adventitious male characters disappearing.†

On the evidence of embryology alone one would suspect that there must be a close functional relationship between the sexual glands and the adrenal cortex; both are differentiated at the same site and apparently from a common embryological basis. One infers that it is through the adrenal cortex that the sex glands bring about the complex series of growth changes which transform the human body at puberty. Through the pituitary mechanism these same glands can play upon the general growth of the body systems.

Tumours of the pineal gland may be accompanied by a series of growth changes almost identical in nature and scope to those following tumours of the adrenal cortex. How such a function has come to be resident in the pineal is an enigma at present—one which future research will probably clear up.‡

The Rôle of the Adrenals in Race Differentiation.—It is scarcely necessary to recall the fact that secondary sexual characters, which are apparently controlled by hormones elaborated in the adrenal cortex, are employed by anthropologists as marks for differentiating one race of mankind from another. The beard and hairiness of body which characterize the European or Caucasian type of man, and also, to a lesser extent, the Australoid type—

* See Keith, *Journ. of Anatomy and Physiology*. 1910, Vol. 44, p. 251; *ibid.* 1913, Vol. 47.

† Prof. T. R. Elliott (personal communication).

On my arrival in New York I visited Prof. Stockard's Laboratory, Cornell University, and found that he had also arrived at many of the conclusions which are given expression to in these lectures. Prof. Stockard has applied the theory of hormones to explain the various breeds of dogs as well as of human beings.

‡ (1) For recent literature on the pineal gland, see Krabbe: *Meddel. fra Rigshosp. Borneafdeling*. 1917. No. 48.

the most primitive of existing races—I look upon as an ancient and characteristic of humanity. In Mongolian and in Negro types the tendency is to a hairless face and body. In this respect these types are more highly evolved than the European. The immature stage, seen in the European youth, is tending to become fixed as the adult stage in the Mongol and Negro. The growth mechanism which determines the woolly hair and thick everted lips of the negro we do not know, but are justified in regarding both of these features as non-anthropoid and of recent origin. Large labia majora, on the other hand, are characteristic of the European type. Prof. Bolk has pointed out that these structures are relatively large in foetal stages of ape and man and that the European has acquired this feature by a foetal becoming an adult character.

The Periods of The Life-Span.—All the evidence at our disposal points to the evolution of higher human races as having been accompanied by a lengthening of the various growth periods which make up man's normal span of life. The more civilized races of man have longer lives than the primitive races and the primitive races than the great anthropoid apes. It is quite apparent, from what occurs in children suffering from tumours of the adrenal cortex, that Nature has a hormone mechanism at her disposal for accelerating or retarding the maturing of the body. The action of the adrenal cortex must be connected with the regulation of the periods of growth and with the span of life. We have further examples of an interference with the normal maturation of the body in the group of cases at present described under the generic term—infantilism. There are forms such as that illustrated by a specimen in the Museum of the Royal College of Surgeons—the skeleton of Crachami—a girl, 8½ years of age, but in size and in many other features similar to a baby only a few months old. Dr. Hastings Gilford has distinguished this form as a foetal type of “infantilism.” Then there is a type illustrated by such cases as that of Jeffrey Hudson who died at 63, and Bornolaski who died at the age of 98. In such cases the span of childhood covers the whole period of life. They may become sexually potent and yet retain a childish size and appearance, their epiphyseal lines remaining open late in life. Again there are those remarkable cases of infantilism to which Hastings Gilford has given the name of Progeria. Such individuals assume an aged appearance while still in their girlhood or boyhood. The exact nature of the growth disorder in these cases is not known, but it is probable that both pituitary and adrenal mechanisms are involved. Compression or atrophy of the pituitary does arrest the growth of the body—produces a condition of infantilism. In the case of progeria that I have examined, the pituitary fossa is relatively small and the neck of the gland is surrounded by a bony ring formed out of the clinoid processes. The atheromatous condition of the arteries, and

the premature appearance of senility suggest that the adrenals may also be involved. The evidence is sufficient to awaken an interest in the rôle played by hormones in regulating the length of life's span.

CONCLUSION

I am well aware of the imperfect nature of the evidence on which I have based a plea for regarding hormones as the agencies which control growth and evolution. Before coming to a conclusion as to whether a *prima facie* case has been made out, I would beg the reader to look narrowly at the position in which all who believe in the evolution of living things are now placed. Darwin gave the law of Evolution an abiding place in Biology; his great difficulty was to explain how a multitude of anatomical elements became simultaneously modified to serve a single definite functional purpose. A study of the growth disorders reveals the fact that the hormone systems, centered in the pituitary and adrenal glands are organized on a functional basis. Hormone systems represent automatic growth mechanism which, like all living qualities, are hereditary and variable. Hormones represent the elements of an automatic system for the control of growth. For this reason new characters do not appear at the end of a developmental stage but early in the growth of the foetus. New characters appear first *in utero*; later they become fixed as a new character in the adult stage. The hormone growth machinery is just such a one as Darwin was in search of. He propounded the elaborate theory of Pangenesis as a substitute.

In these lectures I have been applying the theory to problems relating to the origin and evolution of human races. But the day is certainly coming when it will be made applicable to the realms of Zoology and Botany and provide a real scientific basis for these branches of knowledge.

From the experiments carried out in India by Lt. Col. Robert McCarrison it now seems possible that the substances named vitamins may influence and alter the growth mechanisms of the animal body.

Lastly, from the study of English human remains, representing samples of the inhabitants who have lived in that land at successive periods during the last 4000 years, there is a convincing body of evidence that structural changes are taking place in the jaws, palates and faces of a large proportion of the present population of England. A full knowledge of the hormone system of the human body is likely to reveal not only the cause of these structural changes but also the steps which may have to be taken to combat them.

THE RELATION OF NUTRITION TO TOOTH DEVELOPMENT AND TOOTH PRESERVATION. I.

A PRELIMINARY STUDY OF GROSS MAXILLARY AND DENTAL DEFECTS IN TWO HUNDRED AND TWENTY RATS ON DEFECTIVE AND DEFICIENT DIETS

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The earliest conception of the causes of oral and dental disease may be divided into the systemic or internal and interstitial, and the external or environmental factors.

Hippocrates taught that dental caries was due to "stagnation of depraved juices in jaws and teeth," aggravated by accumulation of food debris.

Galen believed that disturbance of nutrition produced vicious humors of the blood and caused teeth to decay.

John Hunter maintained that teeth were destroyed by internal inflammation and not by external influences.¹

The more modern etiological hypotheses may be divided into the traumatic, inflammatory, infectious, toxic, chemical, chemico-micro-organic, and nutritional.^{2, 3}

Dental defects were conceived to be caused externally by trauma or by decalcification of enamel from acid foods, drinks, and medicines. After the discovery of fermentation, it was believed that micro-organisms, which converted starches and sugars into acids, existed in the mouth and decalcified enamel. Other types of bacteria were said to invade and decay dentin by both fermentative and putrefactive processes.⁴

The saliva was thought to be acid from acid foods or from acid-forming bacteria. Its acidity was also regarded as an expression of lowered blood alkalinity. Inorganic waste in the saliva, which was deposited gingivally, was known to form salivary calculus. This caused irritation of the gums and was said to induce gingival disease.

This conception of local damage by local secretions was applied to solve the etiology of dental erosion. The soluble acid calcium and sodium salts, abnormally excreted by the oral mucous glands, were believed to erode cervical enamel.

The various phases of gingival disease culminating in pyorrhea were said to result from external causes, acting from without inward; the theories of "traumatic occlusion" and of specific infectious invasion may be mentioned as examples. But many of the gingivitis were observed to have their origin in the organism itself and to progress from within outward; examples of this type resulted from scurvy and from massive doses of mercury.

Gingivitis was regarded as the result of "auto-intoxica-

tion," of "excess of uric acid," and of toxic absorption from the gastro-intestinal tract.² But essentially the idea was that of deranged metabolism, "suboxidation," or defective elimination, rather than that of an improperly constituted diet.

The use of bread made of decorticated and degerminated flour was regarded as one of the causes of dental caries. Upon such a diet it was stated that expectant mothers suffered much from dental caries and the child's teeth were so poorly calcified as to be subject to early decay.

Proprietary infant foods were believed to produce enamel hypoplasia, defective dentin and general dental dystrophy.

It was claimed that an excessive carbohydrate diet produced caries in both adults and children and an excess in protein was thought to produce pyorrhea in adults.³

The endocrine glands, particularly the parathyroid and pituitary, were indicted for causing maxillary dystrophy, malocclusion and dental disease, presumably because of their apparent relation to growth and to calcium and sugar metabolism. The oral conditions found in cretins and acromegals were cited as examples.

It had long been assumed that dental caries was caused by excessive sugar in the diet. The incidence of caries was said to develop in the same ratio as sugar consumption. It was supposed to increase the blood sugar which, it was claimed, furnished a fermentable medium for bacteria in the dentinal tubuli. The salivary glands were said simultaneously to excrete glycogen, which fermented on enamel surfaces. So, both locally and systemically, sugar was supposed to attack the teeth.^{2, 3, 4}

The literature on scurvy and rickets has been quoted to show that deficiencies in the vitamins, fat-soluble A and water-soluble B, caused caries of bones and teeth, and pyorrhea.

The statement that "a clean tooth never decays" was used in oral hygiene propaganda and marked results were reported from the practice of oral prophylaxis by patient and operator. Cleanliness is a relative condition and it is now known from a study of those who sedulously

practiced oral cleanliness that caries and gingival disease recur in the so-called "clean mouth."

From this brief etiologic review, which is necessary if this report is to be understood, we can only conclude that oral and dental diseases arise from internal, or systemic, and external, or environmental, causes, or a combination of such causes.

There is a definite relation of oral types: the temporomandibular articulation, maxillæ and occlusion, the tooth form, cusps, roots and attachments, to the form of the gastro-intestinal tract and to food habits.⁴ These must be considered in selecting animals for oral study. The highly specialized and persistently growing molars of the guinea-pig and its equally specialized gastro-intestinal tract are not comparable to those of man, nor is the guinea-pig, with its limited diet of roots and grasses, suited to the study of the omnivorous human diet.

The dog's salivary and gastro-intestinal functions are well known, while comparatively little is known of these functions in the rat. The dog's denture is large and accessible and presents few of the histologic difficulties found in the study of the small molars of the rat. But if dogs are allowed to run, they may obtain supplementary food, thereby disturbing the experimental conditions. If confined, they may develop nutritional disturbances, deformity and disease. The rat thrives under laboratory conditions and is, therefore, the animal of choice for studies of nutrition.

"The rodents include by far the greatest number (nine hundred) and have the widest distribution of any order of terrestrial mammals." Of the sub-family, Muridæ, which included *M. Norvegicus*, (used in these studies), it is said "this genus is the largest in the whole mammalian class, comprising not less than one hundred and thirty species." Such preponderance is founded in great fertility and adaptability, especially to nutritional environment, one factor of which (the rodent denture) is of advantage in obtaining and masticating food.

A composite rodent dental formula consists of incisors $\frac{1}{1}$, canines $\frac{0}{0}$, premolars $\frac{0}{0}$, molars $\frac{1}{1}$, bilaterally. The canines and premolars are missing. The space occupied by these teeth in the usual mammalian denture appears as a diastema (Figs. 1 and II). The incisors are formed and maintained by persistent enamel organs and pulps. Owing to the situation of the mandible and to varying curvature, the mandibular incisors normally articulate posterior to the maxillary incisors.⁵ The glenoid cavity of the temporo-mandibular articulation is longitudinal, rather than transverse. It follows because these teeth are constantly growing, that occlusal surfaces are constantly being worn off—
* * * * *. The elongated temporo-mandibular articulation is important, since it allows the antero-posterior relation in gnawing. The proper regulation of length in the incisors (2.5 mm. average per week) is controlled by their own interaction, a very important factor in the animal economy.¹

All molars of all rodents except the rat grow persistently from constantly functional enamel organs and pulps, and therefore must be continually worn and renewed. They are of the lophodont or hypsodont coronal pattern common to herbivorous animals and are very unlike human teeth. In the rat the molars are completed teeth of the bunodont coronal type common to the omnivora, including man.⁵ They are all erupted by the thirty-fifth day⁶ and are completed apically and attached by fixed alveoli by about the sixtieth day. These twelve teeth are, therefore, comparable numerically and anatomically to the molars of man (Figs. I and II).

According to the period in which defects arise, it should be possible to state approximately whether they are developmental or structural disturbances observed in adult teeth.

The association of two such divergent dental types in the same animal is exceptional in the mammalia. It has a high experimental value, for the effect of nutritional errors may be observed in the persistently growing incisors and compared with that in the fixed molars (Figs. 1 and II).

Of the numerous diets employed for the study of the relation of certain nutritive principles to bone pathology, fifty-seven were selected as affording data which would be especially worth while for the study of the relation of the diet to the development of oral structures. These were so constituted that the effect of small variations in quality of several dietary factors on the maxillæ, teeth and attachments, could be interpreted.

We became interested in the study of the relation of the character of the diet to tooth development and tooth preservation through our observations on the relation of the diet to rickets and related conditions in the rat. It was shown in the latter studies that the skeleton of the rat is extremely sensitive to the composition of the food, especially as respects its content of calcium, phosphate, and fat-soluble A, and to another organic factor which is contained abundantly in cod-liver oil, and less abundantly in leaves of plants, but which occurs only in insignificant amounts in the cereal grains, legume seeds, muscle meats and most other common foods in use in the temperate regions. Faulty relations between these factors induce remarkable deviations from the normal histological structure of the bones, and our studies have shown that the young rat is extraordinarily sensitive to defects in these factors in the diet.

Casual observations had long ago shown us that our experimental animals which fell short of the optimum in their physical development had teeth which were poor as compared with those of animals on diets of excellent quality. It was, therefore, decided to study in a systematic way the teeth of a series of groups of rats which were prepared primarily for the purpose of securing bones for histological examination. This study of rickets and related conditions was a very comprehensive one, embracing a series of diets which were modified in more than seven hundred and fifty different ways. Up to the present time we have prepared and examined about seventeen hundred animals, representing a wide variety of diets, the biological values of every factor of which we have been able to evaluate with a fair degree of accuracy. The detailed study of this series of skulls requires considerable time and will be reported later. In the present paper we present a preliminary study of 220 animals, which were typical of groups restricted to fifty-seven diets variously modified.

It is now possible to study the dietary factors in their relation to tooth development and to permanency of these structures under circumstances far more favorable than hitherto, because we now are in possession of much knowledge concerning the nutritive needs of an animal, and also of the dietary properties of a large number of our more important foodstuffs. Furthermore, the specific

effects of faulty diets of many kinds have been carefully observed, and in the case of rickets we have an extensive knowledge of the chemical causes underlying a pathological syndrome of great significance. In this and future communications we shall present evidence which can be interpreted in no other way than that the developmental factor is an exceedingly important one in determining the quality of the dental structures, and that the composition of the diet is of profound significance both in forming and maintaining good teeth and supporting and nourishing attaching tissues.

For more than twelve years we have maintained our breeding stock of rats on a monotonous dietary regimen, which experience had taught us was more satisfactory in inducing vigorous growth, high fertility, and success in the rearing of vigorous young, than could be attained by feeding table scraps, dog biscuit, bread and milk, or a mixture of grains, forage plants, and of foods of animal origin, where a freedom of selection was permitted the animals. With this diet we have been so successful that we have steadily improved our animals from year to year, not alone through uninterrupted nearly optimal nutrition, but also through rigid selection, year by year, of the most perfect specimens for renewing the breeding stock. We have employed animals from this stock as a standard of excellence with respect to dental structures. The experimental animals were of the same stock (their own progeny), but reared on diets which deviated in some one or more factors from the ideal relationship or composition essential for optimal nutrition. One criterion of the degree of success which we have attained in the feeding of our breeding stock is the nearly complete absence of dental lesions in most of the animals from these controls, even though they were twelve to fifteen months old, or nearly half way through the extreme span of life of this species.

The stock ration consists of a mixture of wheat, maize and rolled oats, 30 per cent each, flaxseed oil meal 10 per cent, whole milk *ad libitum*, fresh cabbage or carrots twice a week. The seed mixture was ground together so finely as to preclude the possibility of the animals picking out and eating the components separately.

The experimental diets employed in these studies are tabulated and described in Tables I and II. For detailed discussions of these diets reference should be made to the charts.

Nutrition studies in recent years have brought to light the fundamental principles upon which quality in foods is to be interpreted. Present day studies are revealing how remarkably sensitive is the protoplasm to deviations from the optimal composition of the diet with respect to several factors. No one suspected until very recently that adherence even for a brief period to an improperly constituted diet could induce changes in the histological structure of any of the body tissues which would be

possible of demonstration by histological technic. Our studies of the effect on the osseous system of changing the concentration and relative proportions between calcium, phosphorus, and one of the vitamins (fat-soluble A) in the food supply have shown that aberrant growth of the several anatomical elements in the bone can be easily induced. This is accomplished without making the deficiencies or excessive concentration of one or another of these nutritive principles greater than is likely to occur in the nutrition of American families at the present time.

It has been abundantly proven that the essential components of an adequate diet for man are the following:—Protein of good quality in sufficient amount. This is equivalent to saying that the protein of the diet must furnish sufficient amounts of each of the eighteen or twenty amino-acids essential for the construction of the tissue proteins. It must contain a source of the sugar glucose. This may be starch, cane sugar, malt sugar, milk sugar or dextrins. It must contain nine mineral elements in appropriate combinations. These are sodium, potassium, calcium, magnesium, chlorine, iodine, phosphorus, sulphur, and iron. These may all be furnished in the form of common mineral salts except sulphur, which must be furnished to the body in the form of the amino-acid cystin, which is contained in most proteins. In addition to the above named substances, the diet must furnish three, and probably four, substances of unknown composition which we designate collectively as vitamins, and specifically as fat-soluble A, water-soluble B, and water-soluble C, and a calcium-depositing factor. There is no experimental evidence in support of the view that man or animal actually requires fat in the food. At present it is not possible to introduce fat-soluble A into the diet without also introducing some fat, so this matter is unsettled.

It will be seen, therefore, that the diet may be remarkably simple in its construction and still meet all the needs of the body. We now appreciate, however, that even a wide variety of foods from certain sources yield diets which are not properly constituted for the maintenance of satisfactory nutrition, either in the growing young or the adult. This appreciation of the damage which may result from taking a diet in which the content and proportions among certain inorganic elements, notably calcium and phosphorus, and the content of one or another of the vitamins, differentiates sharply the present day viewpoint of students of nutrition from that of workers in this field a decade or more ago.

It is especially significant from the standpoint of prevention of oral disease that our knowledge of the pathological conditions resulting from faulty diets is more complete in respect to the development of the osseous system than in respect to any other syndrome. It has been shown experimentally that diets resembling those commonly used in American homes produce profound

alterations in skeletal development. This occurs whether the diet be fed directly to the young as soon as they can be safely weaned, or to the mother during pregnancy or the nursing period. The prevalence of rickets in babies and young children suffices to convince even the most skeptical that we are in ordinary practice actually feeding our infants and children so as to induce malnutrition of this type in very numerous cases. Since the teeth are in a way closely related to the osseous system, it is to be expected that faulty diet should play an important rôle in causing poor development of the dental apparatus.

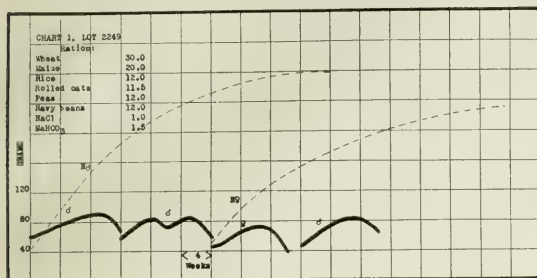


CHART 1.

Lot 2249 was fed from weaning time on a diet of cereal grains and legume seeds (peas, beans), supplemented with sodium chloride and sodium bicarbonate. The latter salt was added because the cereals all contain an excess of acid-forming elements (chlorine, phosphorus, sulphur) over basic elements (calcium, magnesium, sodium, potassium), and the amount added was sufficient to make the diet as a whole slightly alkaline.

The chief fault in this diet was lack of calcium. According to our studies, this mixture of cereal grains and legume seeds contained only about one-fifteenth of the optimal calcium to meet the needs of the growing rat. It contained only about half the requirements of fat-soluble A for this species. The protein content was somewhat below the optimum, both in amount and quality, and the diet could be enhanced by the addition of phosphorus, although the deficiency in this element was not marked.

On this diet the animals grew very little and declined and died after about three months. They were short, stocky, and rough coated. Their bones were poorly developed.

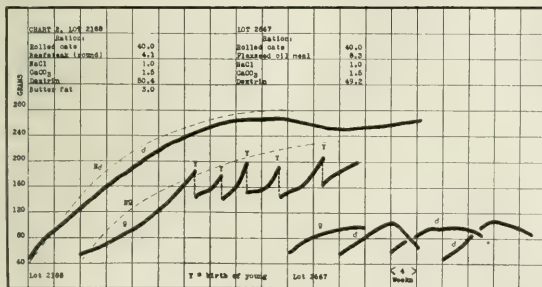


CHART 2.

Lot 2188 had from weaning time a diet containing but 9 per cent of protein of fairly good quality. There were no inorganic deficiencies, since the beefsteak contained phosphorus in liberal amount, and calcium carbonate was added in quantity sufficient

to make the calcium content near the optimum. The 3 per cent of butter fat served as a source of fat-soluble A and furnished enough of this factor to support good growth.

The animals grew fairly well, but never reached the maximum size. Their fertility was high, but practically all the young died. Their coats became rough and they looked old at an early age. The cause of this lay in insufficiency of the protein content of the diet.

Lot 2667. The diet of these animals contained 9 per cent of protein of rather poor quality. It was very low in fat-soluble A and in phosphorus, but contained about the optimal amount of calcium.

On this diet ophthalmia developed in from five to eight weeks because of the lack of fat-soluble A. The animals grew but little, and died after about three months. Their coats were never sleek and they presented a poorly nourished appearance. The bones of these rats were poorly nourished.

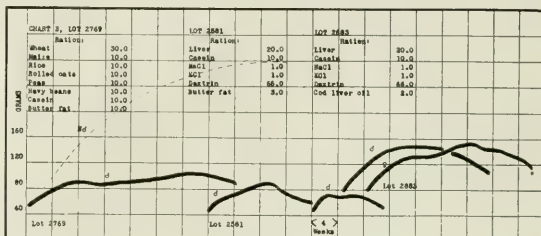


CHART 3.

Lots 2769, 2581, and 2883 may be considered together, since the only deficiency in any of them was lack of calcium. Casein enhanced the quality of the vegetable proteins in Lot 2769. In Lots 2581 and 2883 the casein and liver sufficed as an excellent source of this factor. Butter fat and cod-liver oil furnished fat-soluble A. All other factors except calcium were satisfactorily constituted. When suitable amounts of calcium carbonate are added to any one of these diets, good growth and high fertility are secured. Without calcium additions but little growth was possible, and no young were secured in any of the groups in each experiment. The curves in these charts are those representing the growth of a typical individual in a group.

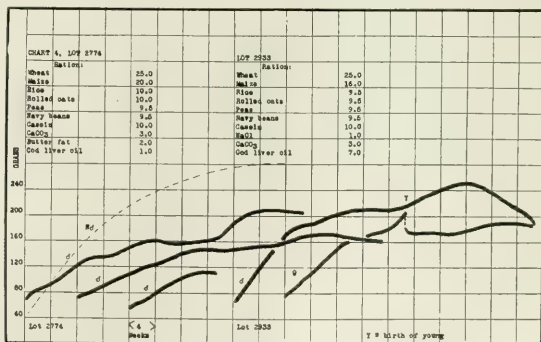


CHART 4.

Lots 2774 and 2933. These two groups had diets which were well constituted except that each contained about double the optimal amount of calcium. For this reason their growth was somewhat depressed and their fertility was interfered with. They had poor coats and did not look well nourished.

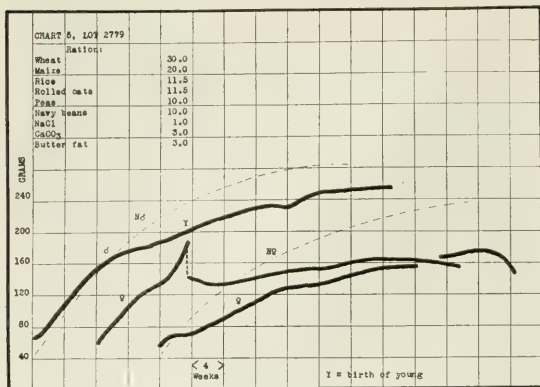


CHART 5.

Lot 2779 had a diet containing double the optimal amount of calcium. The phosphorus content was too low. It should be remembered, however, that a high content of calcium in the diet is tolerated better if the phosphorus content is also high. The content of fat-soluble A is sufficient to enable the animals to grow well, provided all other factors in the diet are properly adjusted, but more of it would have aided the animals in this experiment to tolerate better the excessive intake of calcium and the faulty proportion between this element and the too low phosphorus supply.

These animals aged early, had poor coats and appeared poorly nourished. The fertility was low and all the young born died early. They should be compared with Lot 2775 (Chart 6), where the diet is similar, except that the latter received 10 per cent of a purified protein, casein.

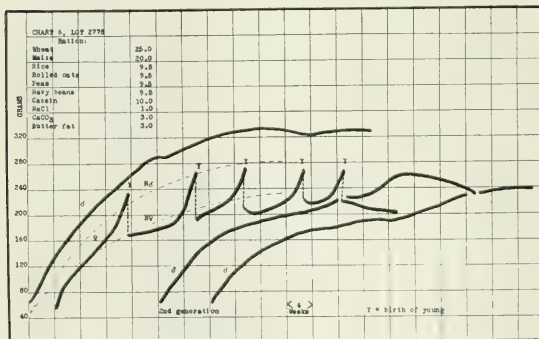


CHART 6.

Lot 2775 had a diet which was well constituted except that the calcium content was too high (approximately double the optimum). They grew well and their fertility was high, but the infant mortality was also high. Several young which were reared were continued on the diet. Their growth was somewhat stunted. An excessive intake of calcium causes early deterioration. It should be appreciated, however, that the optimal calcium intake for the rat is approximately fifteen times the content of this element contained in the cereal grains.

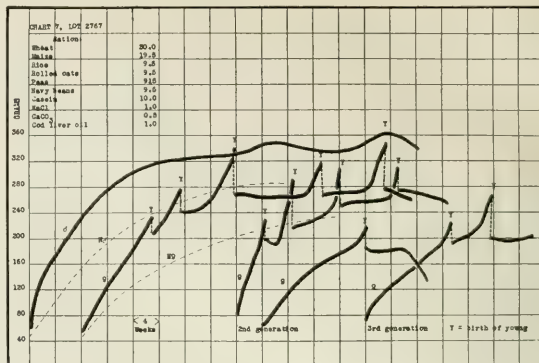


CHART 7.

Lot 2767. This diet was well constituted in every respect except that the calcium content was a little less than half the optimum. The cod-liver oil supplied a high content of fat-soluble A, and this aided greatly in enabling the animals to tolerate the deficiency of calcium in their diet. We believe, however, from experience with other experimental diets, that double this amount (2 per cent) of cod-liver oil would have further contributed to their physiological well-being.

Growth was excellent in this group. The fertility was high and the infant mortality was low. These are the criteria of good nutrition. The second or third generations of animals reared on this diet showed no definite signs of inferiority.

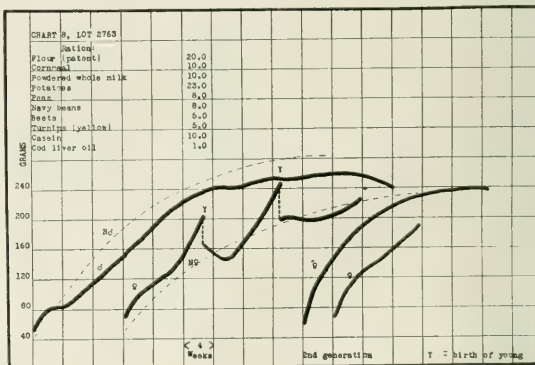


CHART 8.

Lot 2763. The diet of this group presents some very interesting features. It consisted in large part of milled cereal products, tubers and fleshy roots (potatoes), whole milk powder (Merrell-Soule), a purified protein, casein, and cod-liver oil. The last substance was included for the purpose of adding one of the vitamins (fat-soluble A). This diet was very comparable to the better class human dietaries composed of wheat flour, potatoes, meat, and butter. The meat in this diet was replaced by casein. Both are essentially sources of protein and phosphorus.

Growth was only fairly successful, the fertility was rather low, and the infant mortality somewhat excessive. The second generation animals were not so vigorous as their parents.

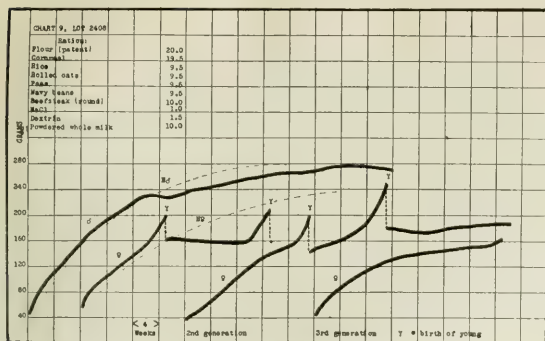


CHART 9.

Lot 2408. This diet was very comparable to such as are usually regarded as of good quality for human beings. It consisted of milled cereals, legume seeds, milk and meat. This type of diet has been extensively studied in our laboratory. It does not contain sufficient calcium or the vitamin fat-soluble A. These faults are sufficient to cause rats confined to the ration to fail to reach the maximum size or to exhibit maximum fertility. When a family is confined to such food supply through several generations, the physical development gradually deteriorates. They show signs of senility rather early and fail to maintain a well-nourished appearance. It is only necessary to introduce more calcium and more fat-soluble A into such a diet in order to change entirely the life history of the individual and of the family restricted to it.

Such diets as are described in connection with Charts 8 and 9, and which correspond rather closely with diets common in American homes, form a very important problem from the standpoint of health and prevention of oral disease. Menus corresponding to such food mixtures look attractive and appear to satisfy the appetite. An example will illustrate this.

BROILED STEAK	MASHED POTATOES
BUTTERED PEAS	COMBINATION SALAD (gelatin, peas, carrots, etc.)
PICKLED BEETS	
BREAD	BUTTER
APPLE PIE	CHEESE
COFFEE	

Such a diet is unsuited for the developing child or for the maintenance of optimal well being in the adult over a long period. Under such a dietary regimen early aging and inefficiency are to be expected.

In order to correct the faults in diets of this type, liberal amounts of dairy products should be included in their various acceptable forms, as butter, cheese, milk, creamed soups, and vegetables of the leafy type in the form of greens and salads. Most important among these are spinach, turnip and beet tops, celery leaves, romaine, collards, lettuce, cabbage, endive, etc. Since our ordinary diets are practically lacking in the antiscorbutic substance (water-soluble C) it is very desirable that some raw fresh fruits or raw vegetables enter into each day's dietary. These may best be used in the form of salads, since this affords an opportunity to introduce further leafy vegetables into the menu in small amounts.

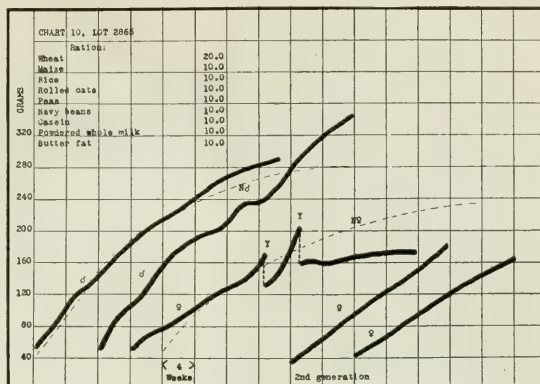


CHART 10.

Lot 2865 had a diet similar to that of Lot 2408, but whole cereals were employed instead of milled cereal products. The chief difference lay in the high content of fat-soluble A (in butter fat) in ration 2865. This improvement was of considerable significance, however. The growth was faster and the animals were in better condition than were those of Lot 2408, which had less fat-soluble A. The diet of Lot 2865 was, however, too poor in calcium, notwithstanding its content of 10 per cent of milk powder. This deficiency was serious enough to make the growth somewhat slower than the maximum rate, and the second generation failed to grow normally.

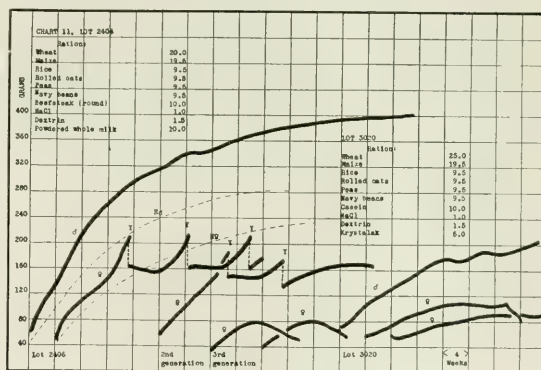


CHART 11.

Lots 2406 and 3020 represent records of two groups of rats on diets of some complexity, but in which the faults were of a two-fold nature. These were insufficient calcium and fat-soluble A. The chief source of both these dietary factors in both diets was the milk powder. In Lot 2406, 10 per cent of whole milk powder, which contains the butter fat, was included. In lot 3020 but 5 per cent of skim-milk powder was included. In other words, ration 2406 was better constituted with respect to both these factors than was ration 3020. The effect of these differences in the two diets is easily seen in the character of the growth curve and the records of reproduction.

Although Lot 2406 grew well and were fairly fertile, the infant mortality was high and they aged early. Such young as grew up were maintained on the family diet but were inferior

to their parents. They were short and stocky, and rough coated, and their span of life was short.

Lot 3020, the diet of which was enhanced with respect to calcium by 5 per cent of skim milk powder, and had no supplementary source of fat-soluble A, was inferior. They were stunted in growth, and infertile, rough coated, and aged early.

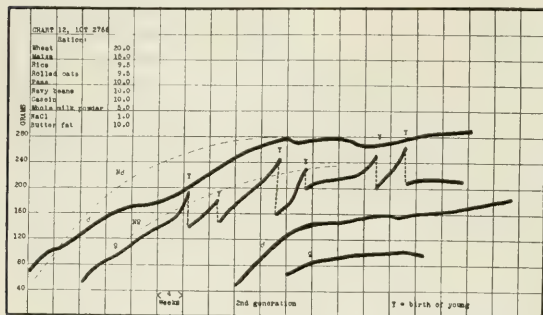


CHART 12.

Lot 2766 had a diet in which there was but one fault of any magnitude, viz., lack of sufficient calcium. It cannot be too strongly emphasized that cereals, legume seeds, tubers, fleshy roots, and meats are inadequate sources of calcium. Five per cent of whole milk in the diet did not furnish enough of this element.

The fairly good showing of these animals with respect to growth and fertility was in no small measure due to the high butter fat content of the diet. Our studies of rickets have made it clear that when the content of calcium in the diet is below the optimal, or there is a serious disproportion between the calcium and the phosphorus content in the food, the presence of a high concentration of fat-soluble A tends in some measure to protect the animals against the effects of the inorganic defect.

These animals grew somewhat slower than the optimal rate. Their fertility was fairly high and the infant mortality was excessive. The few young which were weaned never appeared sleek and well nourished. Their forms were too stocky and their growth stunted.

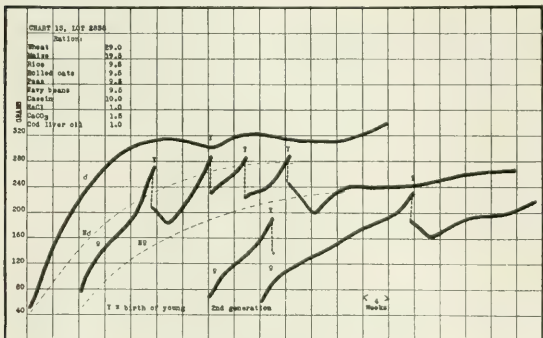


CHART 13.

Lot 2838 illustrates the effect of supplementing diets of the type discussed under Chart 11, Lot 2406, with the optimal addition of calcium in the form of carbonate. Fat-soluble A was furnished by 1 per cent of cod-liver oil. The contrast in the life histories of the two groups of animals under discussion illustrates clearly the importance of having all factors in the

diet properly constituted. In other studies from our laboratory similar contrasts are brought out as the result of making success or failure of groups of animals turn on the quality of factors other than those discussed in this paper. It may be confidently asserted, therefore, that if the diet deviates even in a slight degree from the optimal in respect to any essential factor, the results will become apparent in some degree at some time in the life history of the individual.

Technique. Specimens were prepared by boiling or by maceration (rotting). Of the 220 rats included in these observations, about two-thirds were put on experimental diets at the age of forty to fifty-five days, and were continued on these diets until killed. Many adults were about one hundred to one hundred and fifty days old. No group of less than two rats of the same group was considered, and many groups contained seven upon the same diet. All rats were closely related, since our colony has been inbred without exception over a period of twelve years.

Laboratory observations and the condition of the teeth indicated that the rats had thoroughly masticated the food.

Only gross defects, under the following broad headings, are reported.

Molars I—Caries-like lesions.

" **II**—Loss of attaching tissues and teeth, or both.

Incisors

and

Molars III—Fracture.

Incisors IV—Osteodentin and pulp exposure.

" **V**—Overgrowth.

VI—Loss of maxillary and mandibular areas.

Percentages of defects were calculated from the number of rats and on the diets and number of teeth involved. Caries-like lesions occurred only in the twelve molars, while fractures occurred in molars and four incisors or sixteen teeth in all.

Under the heading "Caries-like defects in molar group," in Table III, is shown the incidence of these defects in rats restricted to the several types of defective diets discussed in the paper.

Attention is called to the remarkably constant percentage of defects throughout the series, while no caries-like defects were observed in stock rats in many generations.

These lesions, which always proceed from without inward, do not result from hypoplastic enamel. They are not absorptions by dental pulp function but have every macroscopic characteristic of caries (Figs. III to VII). That the defects which occurred in the teeth of these experimental rats were comparable to those found in human dental caries is seen in the minute and slowly enlarging initial lesion in any deep enamel sulcus; the rapid dentinal invasion undermining enamel walls; the typical laminated carious dentinal detritus and the final pulp exposure, followed by a subapical bone involvement and alveolar abscesses (Figs. VI and VII).

We believe that further microscopic study, now under way, will confirm these observations. Dental resistance preventing pulp exposure appears to be of the highest

order. Fractures frequently resulted from caries-like lesions, but caries rarely invaded previously fractured surfaces or roots.

The incisors even when hypoplastic or dystrophic are not involved by caries-like defects. Possibly this may be due to (1) the simple tooth form and constant use in gnawing, which prevents food debris retention, (2) the excellent resistance maintained by persistent enamel organs and pulps. If such invasion did occur, complete repair might be expected from the same source.

The majority of lesions arise in distal sulci of the first and central sulci of the second molars. These sulci are much deeper than those in the third molars and caries-like defects may result from food retention (Figs. II, V, VI, VII). Occasionally mesial and distal surfaces are secondarily involved, but, initially, interproximate contact facets are not attacked as in the human (Figs. III and IV). The excellence of contact maintained by attaching tissues may explain this immunity.

The rat's life span of almost three years is said to be comparable to man at the age of ninety. One day, therefore, approximates thirty in the human. No doubt developmental cycles vary greatly, but rats from 120-150 days old may be compared with children between the tenth and thirteenth years.

The teeth of 85 per cent of all school children between these ages are said to be 25 per cent carious. The teeth of 31 per cent of this group of rats were 5.26 per cent carious; an average of twenty-four teeth in children as compared to twelve teeth in rats were exposed to caries-producing agencies. Hence, the disparity between 5.26 per cent for the rat and 12.5 per cent for the child is less than it appears.

The periods during which rats are fed on defective diets, and during which teeth are subject to damage, are important. These vary greatly with different diets and groups, and depend upon the survival of the rat. An example is seen in the extremely rachitic and ophthalmic groups, where they rarely survived over forty days, whereas on the stock diet the animals were vigorous for seventeen months or more.

In the entire group surveyed, the longest period was 510 days for the stock and the shortest 25 days, the average being 260 days. The first and second molars, those generally involved, are fully erupted about the 25th day.

All experimental rats were placed upon these diets at an average age of fifty days. It is a rapid carious lesion indeed which involves a tooth in twenty-five days.

There is no reason to conclude that caries is any more rapid in the rat than in the human. On the contrary, from the resistance inherent in the rodent denture, we believe it to be less rapid. The whole process is relative and can be determined only by study of the individual percentages and diets which are available.

The supposed relation of sugar to human dental caries has been postulated. It is interesting to note that cane sugar does not exist in the fifty-seven diets fed to these groups of rats (See tables I and II).

Certain diets contained 5 or 10 per cent of powdered milk and 5 per cent of beets, but the small amount of milk and beet sugar contained in these is negligible. If these lesions, after further microscopic study, show the well known histopathology of human dental caries (Figs. VI and VII), it might be concluded that cane sugar was not an essential factor in these carious processes.

The diets all contained an abundance of starches, which might perhaps, be regarded as factors.

Under the heading "Loss of attaching tissues and teeth, or both, in molar group," in Table III, is shown the incidence of these defects in rats restricted to the several types of defective diets discussed in this paper.

Attention has been directed to the remarkable stability of the hard and soft attaching tissues of the molars in the rat. The flare of roots to meet occlusal stress is great, if we may judge by occlusal facets. The stout alveolus and well adapted gingival crests all indicate that such teeth cannot be lost without great damage to attachments (Figs. I and II).

It cannot be said that lack of use causes attaching tissue defects. Whether the diet be soft or hard, the molar cusps of a normal rat one year old are abraded like those of a man of fifty years.

Only such gross osseous defects as actually exposed cervical aspects of roots, or finally caused loss of teeth, are reported, and it is assumed that soft gingival tissues had been involved for some time. A microscopic study of these gingival crest lesions should be interesting and will be reported later.

Osseous defects fall into two groups: (1) the atrophic type with progressive, horizontal and regular loss of the alveolar crests, the adjacent cortex being hard, rounded and smooth (Fig. VIII); and (2) the infective type, with irregular, vertical invasion of the alveoli, and localized root exposure, in which the cortex is rough, soft or missing as in true exudative gingival lesions (Fig. IX). In fact, all phases of gingival disease occur as in the human (Fig. X).

Age and the dietary periods must be considered in individual groups in classifying attaching tissue lesions. In man gingival disease generally involves the adult, and thirty-two teeth must be compared with twelve molars in the rat. There are, therefore, 37.5 per cent less teeth exposed to the agencies which induce gingivitis in the rat than in man. The percentage of human gingival disease is not known, but a forty-one per cent involvement of the attachments of the teeth of these experimental animals, many of which were quite young, is significant.

In Table III, under the heading "Fractures in Incisors and Molars" is shown the incidence of this type of injury in teeth, resulting from several types of dietary faults.

A small percentage of fracture (0.024) will be noted in stock rats. This is not unusual in the molars of foraging rodents. Bebb's exhibit (Northwestern University Dental Museum) illustrates these conditions in wild rats. The damage, however, is inconsiderable when compared with the percentages here reported in the group on experimental diets. Such a high incidence of fractures in wild rats would seriously interfere with the nutrition.

Although growing persistently, there are no evidences of repair if incisors are badly fractured. Frequently these teeth are so brittle as to fly to pieces when seized with dissecting forceps or so chalky as to crumble if touched (Fig. XI). Fracture of the incisors is commonly described as existing in rodents where the endocrine glands have been removed or damaged.

Many of the defects in maxillæ and teeth ascribed to disturbance of endocrine function have been produced in these experimental rats solely by defective diets. In animal experiments all dietary factors must be perfectly evaluated before conclusions of any value as to the relation of oral disease to nutrition can be obtained.

One of the conclusions reached by G. V. Black as the result of physical tests of human teeth, was that resilience and resistance to stress rested in the organic matrix and not in the inorganic content of the dentin. That any substance so resilient as the orthodentin of the adult rat should be so modified by diet as to become chalky or brittle is remarkable. This is conceivable in the incisor group where the vascularity of the persistent pulp might modify the dentinal matrix through the tubuli and fibrils; but the causes are not so apparent in the stable pulps and fixed dentin of molars in which the greatest percentage of fracture occurs (Figs. XI, XII and XIII).

Increasing brittleness of human teeth has been frequently observed as senescence advanced. Extremely hard and brittle teeth are common in all life periods of many races, notably among the Italians (Sicilians).

A histologic study of the matrix of such dental types as compared with brittle rat molars would be valuable if the actual diets of the people were known.

In Table III, under the heading "Pulp Exposure and Osteodentin in incisors," is shown the incidence in teeth of these abnormalities in relation to faulty diets.

Pulp exposure in molars is not mentioned, for it is negligible except that which results from caries-like lesions (Figs. V, VI and VII). Exposures which were produced by fracture have already been discussed (Fig. XI). These were quite open, easily detected, and differed materially from exposures here classified.

In normal incisors of the adult rat at least four millimeters of sound orthodentin exists between the pulp apex and grinding surface (Fig. II). If this is not normally renewed, or if it is replaced by osteo-dentin, such defects are significant of failure in function of the persistent pulp (Fig. XIV). In these conditions the

pulp chamber is enlarged in all directions and dentinal walls are thin with consequent shortening of the curvature of roots. The enamel is rarely hypoplastic, though it often becomes mottled or loses its color (Fig. XV). This is evidently a dentinal condition comparable to osseous disturbance, associated with enlarged medullary spaces thin cortices and the formation of osteoid.

In Table III, under the heading of "Over-growth in Incisors" is shown the incidence in teeth of this abnormality in relation to several types of faulty diets. Over-growth is said to result from deviation or protrusion of jaws, or loss of opposing teeth. It is also caused by pulp exposure and disease of molar attachments, both of which produce painful occlusion, and interfere with normal wear of cutting edges, which must equal incisor growth which averages 2.5 millimeters per week.^{7, 8}

Addison and Appleton⁷ report a series of studies on rats, confined in glass jars, presumably to prevent gnawing. The incisors were found to be as much worn by their own interaction as were those in rats eating hard food.

They note the fact that when rats were fed on bread and milk, examples of over-growth in incisors were frequent, but when placed on varied diets, over-growth rarely occurs.

An elongation of the incisors sufficient to prevent contact of grinding surfaces in the molars, which do not grow persistently, may induce malnutrition, if not starvation, in the rat. In the stock rats the elongation was not over 2 millimeters, while in the experimental rats the maxillary incisors were frequently so overgrown that the recurved cutting edges perforated the skull (Fig. XVI).

In Table III, under the heading of "Maxillary and Mandibular Damage" is shown the incidence in jaws of lesions of these types and their relation to several types of faulty diets.

The histopathology of these defects will probably be found to vary from osteoporotic and osteosclerotic states through the phases of bone pathology, similar to those we have described in studies of rickets and related conditions.

The cortex of the mandible and maxilla was thick, roughened, distorted or sclerotic with much repair, or it was canalculated and porous or had completely disappeared (Figs. X to XVII). The medulla was much enlarged and frequently the entire bone was spongy and light with many trabeculæ. The affected areas were remarkably symmetrical and bilateral.

In the mandible, the cortex of each ramus was frequently missing along the incisor root insertions, and root apices were exposed on the external oblique lines (Fig. XVIII).

In extreme cases resorption of the coronoid, condyloid and angular processes occurred, and the rami were fre-

quently so softened that it was questionable whether the mandible could have functioned in mastication (Fig. XVIII).

In the maxilla there was loss of the premaxillary buccal plate, exposing incisor root insertions. Median separation of the premaxillæ and palatal processes occurred, and absorption of the palate bones in the floor of the nares was not uncommon (Fig. XIX).

In both mandible and maxilla, bilateral areas of resorption appeared lingually and buccally in the body of the bones, which could not have been caused by dental disease (Fig. XVIII). These must be sharply differentiated from enlarged sockets and tracts of previous fistulæ in the subalveolar bone induced by gingival disease or alveolar abscess (Figs. VII, IX, XVII).

The defective maxillæ of these rats should be compared by radiographic study with the maxillæ of children suffering from lack of development of the jaws, maxillary and mandibular protrusion and malocclusion, for it is likely that these osseous defects may be correlated (Figs. XVII and XVIII).

It is next to impossible for the orthodontist to stimulate bone growth or establish normal occlusion in the jaws of these children. The teeth may be placed in normal relation by appliances; but as soon as these are removed, the entire denture drifts back into malocclusion.

In adult maxillæ, rarefied areas surrounding the apices of well filled pulpless teeth are discovered by accurate radiography. These are interpreted as alveolar abscesses and oral infective foci, and the teeth removed as a possible cause of focal disease (Fig. VII).

The contents of many such areas are frequently found to be sterile by the most careful bacteriological technique.

It is possible that these rarefactions, which also are often located in parts of the maxillæ other than those adjacent to the teeth, may arise from dietary deficiencies and defects in man as they apparently result in these experimental rats (Fig. XVIII).

In Table III, under the heading "Total Damage—Averaged from the Six Groups of Defects and Fifty-seven Diets," is shown the incidence of damage in relation to specific types of faulty diets. One hundred and six males and one hundred and fourteen females were placed on these diets, and 79.2 per cent of the males and 84.1 per cent of the females were found to have oral defects.

Twenty-seven females reared litters successfully but 92.2 per cent were orally defective.

The bone ash of the hind-leg, pelvis, scapula, and mandible of groups of rats on each of the fifty-seven diets was carefully determined. In stock animals with good bones and teeth, the ash content averaged 66 per cent for hind-leg; 62 per cent for pelvis; 65 per cent for the scapula and 74 per cent for the mandible. In the rats on deficient diets, these percentages were from 5 to 15 per

cent less for all bones, save the mandible, which was constantly from 6 to 8 per cent higher, presumably because of the presence of teeth.

With the exception of the group of diets in which the deficiencies were protein of poor quality and too little fat-soluble A, the fifty-seven diets reported in these experiments were used by two of us in association with P. G. Shipley and E. A. Park in the study of rickets and related osseous pathological conditions.

SUMMARY

As the oral defects herewith reported occurred in the same groups of rats, a comparative summary of the findings in all reports follows:

These reports have shown that the internal structure of the skeleton of the rat could be changed at will by varying the ration which the animals received. In other words, bone is an extremely labile tissue and is readily influenced by nutritional environment.¹⁰

Some of the faulty diets which were studied produced rickets often of an exaggerated type, others caused osteoporosis. Still others resulted in the development of the peculiar lesion, which has been called osteosclerosis.

A study of the effects of these diets on the skeleton would indicate that the growth of the skeleton was dependent on at least three substances; (1) an organic substance present in certain fats which is not identical with the anti-xerophthalmic fat-soluble A; (2) calcium and (3) phosphorus.¹¹

If the organic factor is low in, or missing from, a diet, the structure of the osseous tissue is dependent on the ratio between the above mentioned ions in the diet and the circulating blood.

If calcium is present in amounts equal to, or exceeding, those which would be optimal for growth and function, if all other factors are satisfactory but the phosphate ion is low, rickets is produced.¹² The same disease results when the converse relation exists between the two ions.¹³

In other words, diets which contain optimal or excessive amounts of calcium but are low in phosphorus and the organic factor, produce rickets.

Rickets also results from feeding diets low in calcium and the organic factor, although phosphorus be present in satisfactory amounts.

Diets which are satisfactory except that they are deficient in the organic substance result in osteoporotic but perfectly calcified bone.

Diets which have a comparative deficiency in calcium but are very high in phosphorus and in the organic substance, produce so-called osteosclerosis, with large numbers of small imperfectly calcified trabeculæ.

Diets deficient in calcium alone produce a pseudo-rachitic condition with over-production of osteoid.¹⁴

The studies of diets which contain varying percentages of calcium and calcium phosphate show that the absolute amount of either ion in the diet is of relatively little importance as compared to the ratio which exists between the two. That is, normally calcified bone is produced without regard to the reduction or diminution of either calcium or phosphate in the food, provided the content of the other ion is proportionately augmented or depressed.

CONCLUSIONS

When these findings in the bones are compared with the foregoing table of average damage on all diets, we note the following facts:

The percentage of oral defects was greatest in those rats fed the diets included under the first heading (Tables I and II), i. e., those deficient in protein, calcium and fat-soluble A. The rats on diets low in calcium exhibited the next highest incidence, and those on diets low in both calcium and fat-soluble A had the third percentage of damage.

The diets low in calcium and high in fat-soluble A, those containing low or defective protein, together with low fat-soluble A, and those in which the only deficiency was fat-soluble A, form a middle group producing 11.1 per cent oral defects (Tables I and II). The oral tissues were the least damaged by the diets high in calcium and low in fat-soluble A, those high both in calcium and cod-liver oil, and those low in calcium and in cod-liver oil (Tables II and III).

No caries-like lesions, pulp exposure or osteodentin, attaching tissue or maxillary defects occurred in our stock rats. The 0.36 per cent of defects in stock rats resulted from fracture or over-growth.

The relation of the anti-neuritic and anti-scorbutic substances to oral disease cannot be discussed in this report. A deficiency of the anti-scorbutic substance from the diet of man would no doubt be a factor in the production of oral disease but the rat is able to synthesize this substance.

Polyneuritis, xerophthalmia and scurvy are outspoken expressions of deficiency disease, which, in this country, fortunately are rarely encountered.

It is our belief, however, that severe oral disease may result from diets which are only relatively defective, where the disturbance appears to be out of all proportion to the cause.

In these border-line phases, the dietary defect or deficiency is minute and can only be determined by careful scrutiny of the diet and patient, or of the animals over a considerable period.

It is not possible at this time to name any one deficiency which specifically causes dental or oral dis-

ease; it would appear that any slight variation in the American diet, which always so dangerously approaches the level of dietary deficiency, might become active at any period of lowered resistance or of physical or nervous stress.

The present paper forms a preliminary communication of some of the results thus far obtained. We have not as yet made a detailed analysis of the relative importance of each of the essential factors of the diet in the formation of the teeth, but enough has been learned to point clearly to the diet as the agency of primary importance in determining the quality of the teeth and their vitality, as shown by the effectiveness of their barriers against invasion by micro-organisms.

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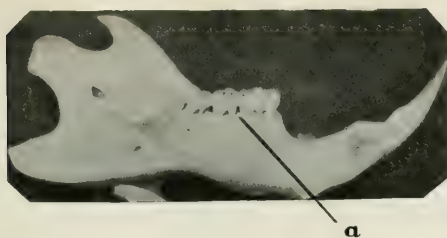


FIG. I.

FIG. I.—The right ramus of a normal mandible of a stock rat. Note the fine type of bone; the excellence and close approximation of the crests of attaching tissues (a), and the bold processes and angles. Enlarged $\times 3$.

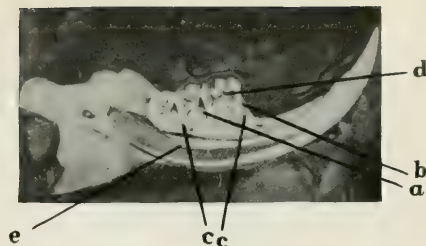


FIG. II.

FIG. II.—Vertical section of the left ramus, of the same mandible, showing the alveolar crests (a); the width of periodontal membranes (b); the flare of roots with pointed apices (c); the finished pulps of molars (d); and the insertion of the incisor with persistently functional pulp (e). Enlarged $\times 2\frac{1}{2}$.

CARIES-LIKE DEFECTS

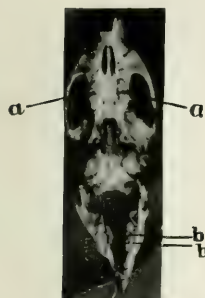


FIG. III.

FIG. III.—Caries-like defects in distal sulci of maxillary molars invading interproximate contact points (a); central sulcus, caries-like defects in mandibular molars (b). Slightly reduced.



FIG. IV.

FIG. IV.—Caries-like defects, maxial and distal, first and second maxillary molars. Enlarged $\times 2\frac{1}{2}$.



FIG. V.

FIG. V.—Deep caries-like lesions in mandibular second molar (a) with pulp exposure. Slightly reduced.

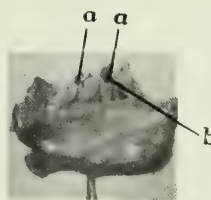


FIG. VI.

FIGS. VI. AND VII.—These illustrate rami of the same mandible. FIG. VII. is a vertical section. Note the large and rapid caries-like lesions involving the right and left mandibular first and second molars (2); the pulp exposures and necrosis (b). Both first molars are dark and necrotic, with periapical abscesses and subapical bone invasion (c) via the open root canals, the alveolar crests (d) being intact. Enlarged $\times 3$.

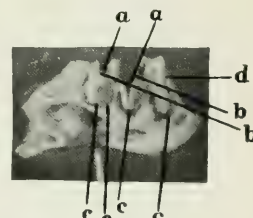


FIG. VII.

MOLAR ATTACHING TISSUE DEFECTS



FIG. VIII.

FIG. VIII.—Lingual and buccal loss of alveolar crests about the second mandibular molar (2), localized and of the atrophic type—simulating gingivitis. Enlarged $\times 3$.

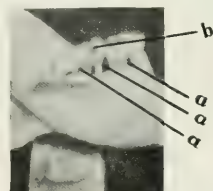


FIG. IX.

FIG. IX.—Illustrating deep invasion of the lingual and interseptal crests (a); teeth tilting and caries-like lesions (b); cortex osteoporosis, simulating true pyorrhea. Enlarged $\times 3$.

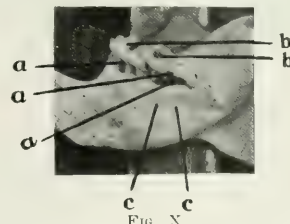


FIG. X.

FIG. X.—Illustrating loss of lingual and buccal crests with deep communicating pyorrhea pockets (a); caries-like lesions (b). The alveolus is deeply and irregularly invaded, and the whole ramus is light, porous and canaliculated (c), simulating rickets. Enlarged $\times 3$.

FRACTURED MOLARS AND INCISORS



FIG. XI.

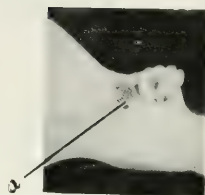


FIG. XII.

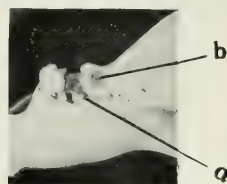


FIG. XIII.

FIG. XI.—Fractured incisors (b), and molars (a). Roots retained. No caries-like lesions. Slightly reduced.

FIGS. XII, and XIII.—Rami of the same mandible: FIG. XII, shows an old fracture (a) with tilting of the much abraded adjacent teeth. FIG. XIII, shows a fracture of distal cusps in second molar (b), and first molar (a). Note that the roots are separating. Enlarged $\times 2\frac{1}{2}$.

PULP EXPOSURE AND OSTEODENTIN, INCISORS

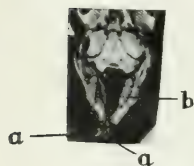


FIG. XIV.



FIG. XV.

FIG. XIV.—Osteodentin gnawing surfaces (a) of mandibular incisors; incipient caries-like defects (b). Slightly reduced.

FIG. XV.—Enlargement maxillary incisor, pulp exposure, (a); pulp chamber enlarged, enamel and apex hypoplastic, (b). General dental dystrophy.

OVERGROWTH, INCISORS



FIG. XVI.

FIG. XVI.—Complete overgrowth maxillary incisors aa which perforate the skull. $\times 3$.

MAXILLARY DEFECTS

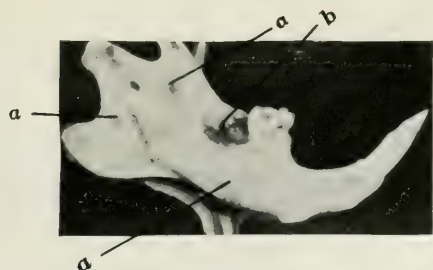


FIG. XVII.

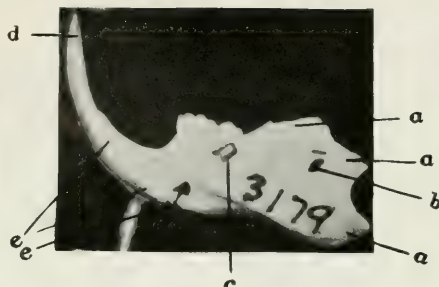


FIG. XVIII.

FIG. XVII.—Left ramus, lingual view adult of rat; resorption areas (a) very thin, porous and canaliculated; old sclerotic lesion in sockets of second and third molars (b), evidently primarily infected via molar roots; compare with (c), Fig. XVIII. Enlarged x 3.

FIG. XVIII.—Left ramus, buccal view of adult rat, showing resorption of coronoid, and condyloid processes also involving the angular processes (a); exposure of incisor root apex (b); loss of cortex and incisor attachments (e); resorption of the basilar alveolar plate not resulting from dental disease (c), for teeth and attachments are normal. Compare with (b), Fig. XVII, over-growth of incisor (d). Enlarged x 3.

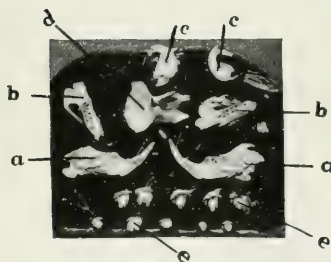


FIG. XIX.

FIG. XIX.—Disintegration of skull, maxillae, and mandible of adult rat. Note angular absorption of the rami (a); the premaxillae containing incisors (c); the maxillary palatal processes (b); the teeth (e), apices absorbed, all detached from loss of attaching tissues. Slightly reduced.

TABLE I

SHOWING PERCENTAGE COMPOSITION AND SOURCES OF THE EXPERIMENTAL DIETS EMPLOYED IN THESE STUDIES.

No. of ration	No. of chart	Wheat	Maize	Polished rice	Roller oats	Peas	Navy beans	Flaxseed oil meal	Casain	Beefsteak	Liver	Milk powder	Butter fat	Cod liver oil	CaCO ₃	NaCl	NaHCO ₃	KCl	Dextrin	REMARKS
2249	I	30.0	20.0	12.0	11.5	12.0	12.0									1.0	1.5			Low calcium, low fat-soluble A, 12.3 per cent of poorly constituted proteins.
2188	II			40.0						4.1			3.0		1.5	1.0			50.4	Low protein (9.0 per cent) of relatively low biological value.
2667	II			40.0				8.3							1.5	1.0			49.2	Low protein (9.0 per cent) of low value Low fat-soluble A.
2769	III	30.0	10.0	10.0	10.0	10.0	10.0		10.0				10.0							Low calcium diet.
2581	III								10.0		20.0		3.0			1.0		1.0	65.0	Low calcium diet.
2883	III								10.0		20.0			2.0		1.0		1.0	66.0	Low calcium diet.
2774	IV	25.0	20.0	10.0	10.0	9.5	9.5		10.0				2.0	1.0	3.0					Twice the optimal calcium content.
2933	IV	25.0	16.0	9.5	9.5	9.5	9.5		10.0					7.0	3.0	1.0				Twice the optimal calcium content, with high cod liver oil.
2779	V	30.0	20.0	11.5	11.5	10.0	10.0						3.0		3.0	1.0				12.3 per cent of protein of relatively poor quality.
2775	VI	25.0	20.0	9.5	9.5	9.5	9.5		10.0				3.0		3.0	1.0				Twice the optimal content of calcium. Twice the optimal amount of calcium.
2767	VII	30.0	19.5	9.5	9.5	9.5	9.5		10.0					1.0	0.5	1.0				About one-third the optimal content of calcium. Slightly less than optimal content of cod liver oil.
2865	X	20.0	10.0	10.0	10.0	10.0	10.0		10.0			10.0	10.0							Low calcium diet.
2406	XI	20.0	19.5	9.5	9.5	9.5	9.5			10.0		10.0				1.0			1.5	Low calcium, low fat-soluble A.
3020	XI	25.0	19.5	9.5	9.5	9.5	9.5		10.0			5.0*				1.0			1.5	Low calcium, low fat-soluble A.
2766	XII	20.0	15.0	9.5	9.5	10.0	10.0		10.0			5.0	10.0			1.0				Low calcium diet.
2838	XIII	29.0	19.5	9.5	9.5	9.5	9.5		10.0					1.0	1.5	1.0				Optimal calcium, slightly below optimal content of fat-soluble A.

* Krystalak.

NOTE: Low calcium signifies a content of calcium derived from the natural foods included in the diet.

TABLE II

SHOWING PERCENTAGE COMPOSITION AND SOURCES OF EXPERIMENTAL DIETS DESCRIBED IN CHARTS VIII AND IX.

No. of ration	No. of chart	Bolled flour	Roller oats	Ground	Whole milk powder	Potato	Polished rice	Peas	Navy beans	Red beets	Yellow turnips	Casain	Beefsteak	Dextrin	NaCl	Cod liver oil	REMARKS
2763	VIII	20.0		10.0	10.0	23.0		8.0	8.0	5.0	5.0	10.0				1.0	Low calcium, and below the optimum in fat-soluble A. Deficient in sodium chloride.
2408	IX	20.0	9.5	19.5	10.0		9.5	9.5	9.5				10.0	1.5	1.0		Low calcium, and below the optimum in fat-soluble A. Approximately the optimal content of sodium chloride

TABLE III

TABLE SHOWING PERCENTAGE INCIDENCE OF DENTAL DEFECTS IN RATS RESTRICTED TO DIETS OF DIFFERENT CHARACTERS

No. of diets studied	Caries-like defects in molar group	Loss of attaching tissue and teeth, or both, in molar group	Fractures in incisors and molars	Pulp exposure and osteodentin in incisors	Over growth in incisors	Maxillary and mandibular damage	Percentage of total damage		Calcium per 100 grams ration	Phosphorus per 100 grams ration
1....STOCK RATION.										
No defects of a significant magnitude. Our breeding stock of rats has been confined to this diet through a period of twelve generations without any deterioration in vitality. During this interval they have improved physically because of the selection of exceptional individuals for breeding.....										
	0.0	0.00	0.02	0.00	1.10	0.00	0.36			
7....DIETS LOW IN CALCIUM BUT RICH IN FAT-SOLUBLE A.										
A diet typical of this group is described in Chart X, Lot 2865. Factors other than calcium in this diet were of nearly optimal quality.....										
	1.18	20.81	2.59	19.08	8.31	19.04	11.66	Lot 2865—	0.1348	0.4110
6....DIETS CONTAINING EXCESSIVE CALCIUM, AND WITH A SATISFACTORY CONTENT OF FAT-SOLUBLE A.										
In this group are included animals fed diets similar to or identical with those described in Chart V, Lot 2779, and Chart VI, Lot 2775. The latter differed from the former in that it contained 10 per cent of casein, and therefore furnished 0.08 grams of phosphorus per hundred grams of ration more than did diet 2779. This modification in the phosphorus content of diet 2775 improved the growth curves, and resulted in better nutrition than would the same food mixture without the added casein. This difference was not due entirely to the added phosphorus, but in part at least to the improved amino-acid supply resulting from the addition of casein. Notwithstanding these differences in the two diets, the effects on the dentition of the animals was so similar that the six diets corresponding to these types are grouped together here. We are now studying in detail the many specific problems which are suggested by these preliminary results.....										
	1.73	33.38	3.28	0.00	0.00	12.50	8.48	Lot 2779— Lot 2775—	1.2530 1.2482	0.3459 0.4053
5....DIETS LOW IN CALCIUM, LOW IN FAT-SOLUBLE A, AND PROTEINS OF RELATIVELY LOW BIOLOGICAL VALUE.										
Growth of young rats on diets of this class is very poor (Chart 1, Lot 2249). All diets of this class in which the proteins are derived solely from cereal grains and legume seeds are below the optimal in the quality of the amino-acid mixture which they yield. This means that the proteins are not so effectively transformable into body proteins as is possible with proteins of better quality										
	2.88	35.50	4.70	37.50	17.85	45.20	23.93	Lot 2249—	0.0216	0.3631
6....DIETS CONTAINING EXCESSIVE CALCIUM, AND CONTAINING SEVERAL PERCENTAGES OF COD LIVER OIL, OR OF COD LIVER OIL AND BUTTER FAT.										
Diets 2774 and 2933, Chart IV, are typical of those fed to animals classed in this dental group. The protein content was of good quality, and was abundant in amount. The calcium content was about twice the optimal. (The Optimal content is about 0.64 per cent calcium, equivalent to about 1.5 per cent of calcium carbonate added to a mixture of cereal and legume seeds). The content of fat-soluble A in these diets was very liberal in terms of the nutritive requirements of the rat.....										
	3.00	16.70	4.48	4.16	0.00	6.93	6.21	Lot 2774— Lot 2933—	1.2482 1.2476	0.4053 0.3982
7....DIETS LOW IN FAT-SOLUBLE A AND LOW IN PHOSPHORUS. THEY CONTAINED NINE PER CENT OF PROTEIN OF RELATIVELY LOW BIOLOGICAL VALUE.										
A typical example of diets of the type under consideration is Lot 2667, Chart II. They were so deficient in fat-soluble A that the animals developed xerophthalmia within a few weeks and their lives were cut short. The skulls examined were, therefore, all of animals which were chronologically young, although they showed physiological deterioration. This is reflected in the high percentage of molar defects in animals but three to five months old....										
	3.12	19.74	8.48	5.00	10.00	18.50	10.80	Lot 2667—	0.6668	0.2214

TABLE III--Continued

TABLE SHOWING PERCENTAGE INCIDENCE OF DENTAL DEFECTS IN RATS RESTRICTED TO DIETS OF DIFFERENT CHARACTERS

No. of diets studied	Caries-like defects in molar group	Loss of attacking tissue and teeth, or both, in molar group	Fractures in incisors and molars	Pulp exposure and osteodentin in incisors	Over-growth in incisors	Maxillary and mandibular damage	Percentage of total damage		Calcium per 100 grams ration	Phosphorus per 100 grams ration
6....DIETS LOW IN CALCIUM, BUT OTHERWISE WELL CONSTITUTED.										
The fat-soluble A content was abundant. The protein was in all cases of very good quality. The calcium content was so low as to cause in some groups stunting of growth, and early deterioration and death. (See Chart III, Lots 2769, 2581, 2883, and Chart XII, Lot 2766). In other diets small additions of milk powder raised the calcium content sufficiently to permit the animals to grow at a subnormal rate. Notwithstanding their capacity to grow, the animals on this latter class of diets (Chart X, Lot 2865, and Chart XI, Lot 2406), were physically inferior to animals on better diets. The high incidence of molar defects in these animals is an index to their nutritional instability										
	3.65	57.90	4.45	14.57	6.25	39.90	20.12	Lot 2769— Lot 2581— Lot 2883— Lot 2766— Lot 2865— Lot 2406—	0.0480 0.0010 0.0010 0.0904 0.1348 0.1356	0.3868 0.4000 0.4000 0.3929 0.4110 0.4301
7....DIETS LOW IN PROTEIN (ABOUT 9 PER CENT) OF RELATIVELY LOW BIOLOGICAL VALUE. THEY WERE NOT FAR FROM THE OPTIMUM IN THEIR CONTENT OF FAT-SOLUBLE A.										
An example of the diets in this group is given in Chart II, Lot 2188. The only pronounced defects in these diets was in the protein moiety. The percentage of dental defects in rats restricted to these diets was so high that we have undertaken a comprehensive study of this factor in relation to tooth development and tooth vitality										
	4.74	9.47	0.08	20.82	7.72	19.71	10.91	Lot 2188—	0.6371	0.1753
7....DIETS LOW IN CALCIUM, AND BELOW THE OPTIMUM IN FAT-SOLUBLE A.										
The calcium content of the diets in this class stood intermediate between those which derived all their content of this element from cereal grains and legume seeds, and those in which approximately the optimal content of calcium was added in the form of calcium carbonate, viz., 1.5 per cent CaCO ₃ . Typical examples of these diets are shown in Chart VII, Lot 2767, and Chart VIII, Lot 2763. All factors other than protein and fat-soluble A were well constituted										
	5.21	17.70	2.88	2.14	3.38	0.00	5.25	Lot 2767— Lot 2763—	0.2503 0.1817	0.4217 0.3713
6....DIETS LOW IN CALCIUM, AND WITH FAT-SOLUBLE A DISTINCTLY BELOW THE OPTIMUM.										
The milk powder contained in these diets was not sufficient to introduce enough calcium to do more than enable the animals to grow at a rate which would seem to indicate fairly good nutrition. Actually, we know, from the results of our observations of the life histories and reproductive capacity of such animals, that they were in a state of nutritive instability. Their span of life is distinctly below the normal, and their infant mortality was high. The young were inferior in appearance and the families tended to die out within two or three generations. The percentage of dental defects was high.										
	5.26	14.94	1.88	37.45	11.10	13.83	14.00	Lot 2406— Lot 2408— Lot 3020—	0.1356 0.1363 0.0936	0.4301 0.3397 0.4438

TABLE IV

SHOWING THE PERCENTAGES OF ANIMALS EXAMINED, WHICH SUFFERED FROM SPECIFIED DENTAL DEFECTS.
(Data Based on Observations on 220 Rats)

Caries-like defects in molar group	Loss of attacking tissue and teeth, or both, in molar group	Fractures in incisors and molars	Pulp exposure and osteodentin in incisors	Over growth in incisors	Maxillary and mandibular damage	Total damage averaged from all groups of defects
31.0	41.8	29.5	22.7	20.0	25.0	Males—79.2 Females—84.1

STUDIES ON EXPERIMENTAL RICKETS. XX. THE EFFECTS OF STRONTIUM ADMINISTRATION ON THE HISTOLOGICAL STRUCTURE OF THE GROWING BONES

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The effect of strontium salts on the animal organism has been studied by chemical and histological methods, chiefly to ascertain whether or not salts of strontium could replace lime salts in the skeleton. The therapeutic use of strontium bromide for epilepsy and kindred nervous disorders has stimulated pharmacologic studies of the toxicity of strontium salts.

The most elaborate investigation of the effect of strontium on bone development is that of Lehnerdt.¹ His conclusions may be briefly summarized as follows: (1) Strontium cannot replace calcium physiologically. (2) Strontium greatly stimulates the formation of osteoid tissue, and tends to repress the resorptive processes in the bone. (3) Strontium stimulates the osteogenetic cells.

Lehnerdt found that the feeding of moderate amounts of strontium with a liberal provision of calcium did not lead to pronounced deviation from the normal osteogenesis. If, on the other hand, strontium was added to a calcium-poor diet, the skeleton grew abnormally. The effect of the feeding of strontium was inversely proportional to the content of calcium contained in the diet.

The stimulating action of strontium on the osteogenetic tissues makes itself most evident at these points at which, under normal conditions or with a calcium-poor diet, there is greatest tendency to resorption, *viz.*, in the oldest parts of the spongiosa; at the junction of the metaphysis and the diaphysis; and on the outer surface of the medullary cavity. In these places strontium caused over-production of osteoid tissue. Lehnerdt believed that there was no abnormal formation of osteoid in the growing bone if an animal was partially deprived of calcium. He thought that only so much osteoid tissue was formed as could be calcified by the limited amount of calcium that was available. The development of new bone under these conditions was accompanied, however, by an increase in the rate of resolution of old bone substance, so that a condition of osteoporosis developed, and a fragile bone was formed, the osteoid of which was produced in so limited an amount that it could be completely calcified. He thought that calcium salts were borrowed from the old, well-calcified parts of the bones to construct new bone in the growing region, and that the thin trabeculae were the result of forced economy in the use of calcium.

Lehnerdt believed that calcium, was, therefore, a regulator of the growth of the organic elements of the bone and that it also, impregnated the osteoid tissue in the form of insoluble salts. He considered that strontium possessed the same property as calcium, of stimulating the development of osteoid tissue, but was many times more effective in this direction. He felt that strontium, could not be used to impregnate the osteoid tissue in the form of insoluble salts in anything like the degree that calcium could, and therefore the osteoid formed under its influence remained soft. The deposition of strontium is a secondary phenomenon, which occurs as a result of a too meager supply of calcium to the bone. Strontium, Lehnerdt believed, can be absorbed only at the expense of calcium.

Lehnerdt fed his dogs strontium phosphate, adding this to a diet of muscle meat and fat. He used therefore, a diet which

was not only very poor in calcium (except when calcium phosphate was added), but was very high in phosphorus. He did not appreciate the significance of this fact.

Lehnerdt found that the bones of animals fed on the above diet showed in various degrees a pathological condition which resembled rickets, in that it was characterized by over-production of osteoid tissue. It differed from rickets, however, in many important respects: (1) The line of junction of the epiphyseal cartilage with the shaft was quite regular. The cartilage neither persisted as it does in rickets, nor was it irregularly destroyed by blood vessels from the shaft. (2) The marrow cavity was nearly or quite obliterated by large numbers of trabeculae which were made up of osteoid tissue, with little or no calcified bone in them. He felt that this lesion was brought about by an enormously increased production of osteoid tissue ("apposition") and the complete or nearly complete suppression of the resorption which goes on in the course of normal osteogenesis. (3) The gross deformity of the bones differed from that caused by rickets.

Lehnerdt was able to induce the condition in the fetus *in utero* and also in the bones of pups nursed by a strontium-fed mother for 32 days, as well as in adult animals (dogs and rabbits). The affected bones grew in length and in diameter. The cartilaginous ends of the bones and the junction of the shaft with the head of the bone were not affected. The cartilages were calcified almost or quite completely. The line of junction of the epiphysis with the shaft was regular and sharp. The ends of the shaft were composed of a mass of trabeculae. Each of these was surrounded by or composed entirely of osteoid tissue. Some of these osteoid trabeculae contained rests of calcified intracellular matrix of cartilage. Others were calcium-free. The marrow cavity in the growing region was completely obliterated by the osteoid trabeculae (sclerosis). In the shaft the marrow cavity was reduced in size by over-growth of osteoid tissue in the cortex, and the persistence of osteoid spongiosa, which should have been absorbed. Lehnerdt believed that one month after birth the bone which had formed the cortex of the bones at birth still persisted unchanged. Growth of the shaft in diameter was carried on by the development of masses of osteoid tissue beneath the periosteum.

The bones of adult and nursing animals were not greatly deformed in spite of the severity of the lesion. The ribs showed a change at the costo-chondral junctions which differed from the rachitic change in man in that there was not much swelling of the junction or deformity of the thorax. The ends of the ribs did show a bullet-shaped swelling, of a whitish color, at the growing end of the shaft which corresponded with the region of "sclerosis." Wrist and ankle joints were not markedly enlarged.

The pathological picture of this strontium sclerosis was much exaggerated in the bones of pups of strontium-fed mothers.² The bones of these animals were very badly deformed and had practically no medullary cavity.

We have studied the reaction of young rats to strontium to determine the relationship of "strontium sclerosis" to rickets, and to determine, if possible, the relation of the alkaline earth ion to the production of the rachitic lesion. Our studies of the effect of strontium on grow-

ing bones were carried out in the light of a wide experience in observing the manner in which specific starvation for calcium or for phosphorus, deviation from the optimal ratio between calcium and phosphorus, and the effect of deprivation of liberal provision of an organic substance associated with certain fats, notably cod-liver oil, modified the histological structure of the bones. We are, therefore, able to interpret our results from a more comprehensive viewpoint than could Lehnerdt, who failed to appreciate the importance of an excessive amount of phosphate together with a deficient amount of calcium in the diet in modifying the structure of the bones of his animals. The significance of this will appear later.

It is essential that the character of the diet which we used in these experiments be understood, in order to appreciate the influence which we assign to strontium in modifying the structure of the growing bones. Diet 2581, which we employed as a basal one in this study consisted of

	Per cent
Liver (cooked and dried)	20.0
Casein	10.0
KCl	1.0
NaCl	1.0
Dextrin	65.0
Butter fat	3.0

one hundred grams of this diet contained .0008 gm. of calcium, and .4060 gm. of phosphorus. The weight ratio of calcium to phosphorus was 1:507.5. The atomic ratio of calcium to phosphorus was 1:654.2.

There was but one fault of a demonstrable magnitude in this food mixture (for the rat), viz., lack of calcium. Our studies have convinced us that the optimal content of calcium for the growing rat is about 0.64 per cent (Ca.). The optimal phosphorus content has not been established so accurately as has the optimal content of calcium, but provisionally we have deduced from our studies that, other factors being satisfactory, about 0.40 per cent (P) represents the minimal content on which optimal development can take place. The proteins of this diet were of excellent quality, and were abundantly supplied (24.8 per cent). Both the butter fat and the liver are rich in fat-soluble A. Liver is rich in water-soluble B. The needs of the animals for sodium, potassium and chlorine were supplied by the additions of potassium and sodium chlorides noted. When calcium was added to this diet in the form of carbonate to the extent of 1.5 per cent of the food mixture, rats were able to develop in a perfectly normal manner. This is shown by the records of Lot 2162, Chart 1. Without the addition of calcium no growth was possible on this food mixture, as is illustrated by the records of Lot 2581, Chart 2.

A study of the effects of this diet, with and without

calcium additions, affords an interesting illustration of the importance of a proper balance between the calcium and phosphorus in the diet. When the formula given above was fed as the sole source of nutriment, the skeletal development was very abnormal.³

The diet without calcium addition failed to induce appreciable growth. The hair of the rats was rough and irregular in length. Their skulls were thin and easily compressible. The incisor teeth were brittle, yellow, and in most instances easy to extract. There was never any evidence of ophthalmia. The epiphyses of the long bones, especially at the wrist and knee, were enlarged. The shafts of the bones were slender. The thoraces were much flattened from side to side, slender, and marked by deep grooves along the costochondral junctions. The junctions of ribs and costal cartilages were enlarged, and there was marked angular deformity of the thorax. The degree of angular deformity exceeded that found in the ribs of rats fed diets low in fat-soluble A and phosphorus. The thymus was atrophied. The spleen was frequently greatly enlarged. There was little, if any, subcutaneous or visceral fat. The testes were atrophic. The long bones cut easily. There were, as a rule, no fractures to be found in any of the bones except the ribs, which were usually studded throughout their length with the calluses of healing fractures.

The bones of rats which have been on diet 2581 were in a pathological condition closely allied to rickets, but which varied in some respect from that disease as it is commonly seen in children. The bones resembled those of rachitic children in that they were characterized by the complete or nearly complete failure to form a provisional zone of calcification. They had a pathological metaphysis between the shaft and the epiphysis of the bone, and there was a great over-production of osteoid tissue with the consequent deformity of the skeleton. The epiphyseal disc of cartilage was somewhat widened, and was invaded to a slight degree by blood vessels from the diaphysis. The cartilage cells underwent degeneration and metaplasia into osteoid and the bone marrow in the growing region of the bone was entirely replaced by newly formed connective tissue. On the other hand, the condition varied from human rickets as it is ordinarily seen in several ways. The cartilage of the epiphysis was not persistent to the extent usually seen in human rickets, or in animals which have received diets which are high in calcium, low in phosphorus, and in a substance which is present abundantly in cod-liver oil. The cartilage was not as much disrupted by vascular invasion, and the columnar arrangement of the proliferative zone was not greatly disturbed. The metaphysis was narrow, and its entire arrangement was much more regular than that usually seen in human rickets of a grade sufficiently severe to induce an equivalent amount of skeletal deformity. Trabeculae were present in extraordinary numbers in the spongiosa. The marrow cavity was encroached upon and much narrowed by the cortex, which was greatly thickened and consisted of a sponge-like formation of partly calcified trabeculae. Signs of increased and active resorption were seen throughout the shaft, especially in the cortex and in the older part of the spongiosa. The pathology of these bones has been described at length in another communication.³

The condition shown by these bones was somewhat analogous to that described by Schmorl, and to which he gave the name *pseudo-rachitic osteoporosis*. It was apparently a condition which is on the borderline between the pathological picture of rickets as it is usually seen, and the lesion which is known as osteosclerosis. The latter condition may be induced by feeding certain diets containing

an abnormally large amount of phosphorus. We have shown, however, that rickets in the rat, or at least a condition which is identical with rickets from the histological point of view, may be induced by feeding diets which are low in calcium, but which contain an approximately normal amount of phosphorus.⁴ A pathological picture, identical with human rickets as it ordinarily occurs, also results from the ingestion of diets which are high in calcium but low in phosphorus and fat-soluble A.⁵ The disproportion between these inorganic salts in either direction must be, however, within certain very definite limits, if the bone lesion produced by them is to be identical with the rickets of children. By varying the proportion in which these ions exist in the animal's diet bones may be produced which are either normal, rachitic, or sclerotic, but it must be appreciated that the line of demarcation between the lesions is not sharply cut, and that transition states may occur between conditions of rickets and osteosclerosis, between rickets and normal bone and between normal bones and sclerosis. We have suggested in another place that the rachitic condition which is accompanied by tetany may sometimes be a type different from rickets as it is commonly seen and which tetany never complicates. We have suggested moreover, that there may be in children, as in rats, transition forms of rickets, such as rickets of the so-called healing type.⁶

In order to demonstrate the effects of strontium in the absence of calcium on the growing bones, we employed Diet 2581, the effects of which have been described above, with the following additions.

Diet 3152.

Liver	20.0
Casein	10.0
NaCl	1.0
KCl	1.0
SrCO ₃	2.2
Butter fat	3.0
Dextrin	62.8

This diet contains an amount of strontium which was the atomic equivalent of 1.5 per cent of calcium carbonate in the diet, or the equivalent of 0.6 per cent of the element calcium. The dietary properties of this food mixture, aside from the strontium contained in it, have been described above.

The results of feeding this diet to young rats throw some light on the question as to the possibility of strontium being capable of replacing calcium in a physiological sense. Without the addition of either calcium or strontium young rats on this diet grow scarcely at all. With the optimal calcium addition they grow normally. Without calcium but with strontium addition equal to 2.2 per cent of the food mixture, they are able to reach twice or three times their initial weight in most cases. This is especially true when cod-liver oil is included in

the diet, but the cod-liver oil did not exert a protective effect on the bones in the sense of preventing the abnormalities in histological structure. It will be recalled that cod-liver oil is extraordinarily effective in preventing the abnormal development of bones characteristic of rickets and related conditions, when these are brought about by unfavorable relations of calcium and phosphate in the food, an insufficiency of fat-soluble A, or the second organic factor associated with certain fats which our experimental data seem to demonstrate to play an important rôle in bone growth.

Animals confined to diet 3152 developed a strange condition after from 25 to 35 days, manifested by peculiarities of gait and of sitting posture. In the early stages they sat with the back rounded in an abnormal manner, and with the hind legs held in a loose and irregular fashion, as if they were suffering from a partial loss of muscular control. As the condition grew worse, the hind legs become nearly useless in walking and were dragged behind the animal. Cod-liver oil tends distinctly to protect the animals against this loss of muscular control.

Autopsy. These animals were all fairly well grown. Their coats and eyes showed nothing abnormal. The skulls were very soft. The teeth were slender, white, and fractured easily. In some animals even the molars were loose. Occasionally the incisors were broken during life. The thymus and testes were always normal. The spleen was always much enlarged. No other visceral abnormalities were found. Often there was no deformity of the thorax but sometimes the chest wall was slightly sunken along the line of the costo-chondral junctions. The shafts of the ribs were enlarged behind the junctions with the cartilages. There were sometimes healed fractures of the shafts of the ribs, but often none were to be found. The long bones showed very marked changes. Their extremities were greatly enlarged so that the wrist, knee and ankle joints appeared to be swollen. The swellings were snow-white and were so soft that free movement in all directions was permitted as though the bones were fractured on the shaftward side of the epiphysis. The extremities of the bone cut very easily, but there was a considerable resistance and grating when the shaft was cut.

On section the epiphyseal centers of ossification were small and the cartilage was much increased in depth. The junction of the cartilage with the shaft was irregular and the cartilage was invaded by large tufts of blood vessels. A broad zone of white tissue (the metaphysis) separated the cartilage proper from the shaft. The cortex of the shaft was thickened somewhat as the result of the growth of osteoid tissue. The shaft contained a relatively small number of trabeculae, each surrounded by a zone of osteoid tissue.

Histological. On microscopic examination the most striking finding was the over-growth and persistence of the epiphyseal cartilage and the breadth of the metaphysis. The zone of resting cartilage was abnormally wide and the matrix between the cells was abnormally abundant in this region. At the beginning of the proliferative zone reproductive activity of the cartilage cells was marked, so that the abundant matrix was replaced by cells which were crowded together in a dense mass which soon lost any semblance of columnar arrangement. The proliferative zone was in many places from 20 to 30 cells in depth. At the junction of the proliferative zone with the resting cartilage the cells had a fusiform or crescent shape, and the cytoplasm and cell capsules stained intensely with basic dyes. The older cells, however, soon became globular. Unless contact was established with the blood vessels of the shaft, they retained their affinity for basic dyes. Intense activity of the

cartilage cells was apparently the first manifestation of the onset of the lesion. Calcification of the cartilage did not occur. The cartilage was invaded and destroyed in an irregular way by single capillaries and by large tufts of vessels from the shaft. Large tongues of cartilage persisted in the metaphysis. The cartilage cells degenerated or underwent metaplasia into osteoid corpuscles as soon as they came into contact with the metaphyseal blood supply. Metaplasia was often preceded by active division of the cells (Figs. 2 and 3), but the daughter cells did not grow, so that the bone corpuscles derived from the cartilage were very much smaller than the parent cells.

The initial step in the processes of metaplasia or degeneration was loss of the ability of the cell capsule and cytoplasm to take up basic dyes. In the vicinity of the blood vessels many of the cartilage cells became degraded into cells of the fibroblast type. Their nuclei became pyknotic and they assumed a spindle shape. (Fig. 2). In this condition they might become embedded in osteoid tissue where degeneration might go on until the entire cell disappeared and nothing remained to indicate that it had ever existed except the space occupied by it in the osteoid. Sometimes small groups of cartilage cells or single elements could be found embedded unchanged in a mass of osteoid in the metaphysis. Cartilage cells in the long tongues which persisted in the metaphysis frequently reverted to the form seen in the beginning of the proliferative zone, and became flattened and fusiform in shape. So long as these cells were protected by osteoid or other cartilage cells from the influence of the blood vessels, they retained their identity and their affinity for basic stains. At the border of the cell masses, however, the cytoplasm stained like osteoid tissue. As the blood vessels approached the cartilage cells, the cells divided rapidly within their capsules into numerous small daughter cells, and these became embedded in osteoid and indistinguishable from osteoid corpuscles (Fig. 3).

The metaphysis which formed in these bones was deeper than that in any other bones we have examined (Fig. 1). It consisted of osteoid trabeculae and cartilage cells, unchanged, or in all stages of degeneration and metaplasia. It contained numerous blood vessels surrounded by fine connective tissue reticulum supporting a few marrow elements. Some of these vessels were very large. Finally, the masses of unchanged cartilage described above were present. The trabeculae of the metaphyses usually contained no trace of calcium, but occasionally one was found with a bit of calcified intercellular matrix in its center.

The trabeculae of the spongiosa were not numerous, and were usually well formed and straight. Each was more or less completely surrounded by a border of osteoid tissue (Fig. 4). The osteoid corpuscles were small. The osteoblasts surrounding the trabeculae were in a continuous layer and were flat cells like blood vascular endothelium. The calcified cortex was slender and dense and was covered by osteoid tissue which was traversed by numerous blood vessels (Fig. 1). The medullary cavity was very little narrowed. The bone marrow cells were normal.

The junction of the metaphysis with the shaft was marked in these bones by a line of calcified intracellular substance embedded in osteoid tissue (Fig. 1,r). This line showed the situation of the epiphyseo-diaphyseal junction at the time when the first effects of feeding the strontium-containing ration manifested themselves. No signs of resorption of bone were evident.

Briefly then, the effect of feeding this ration to rats is to produce in the skeleton persistence of the cartilage, and increased formation of osteoid tissue. A pathological metaphysis is formed and the epiphyseo-diaphyseal junction is rendered very irregular. Resorption of the bones is checked during the administration of the

ration. Clinically the condition is characterized by deformities of the skeleton resembling those which result from rickets in man and by partial or complete paralysis of the extremities.

We cannot discuss the cause of the paralysis at this time. We cannot say whether it is dependent on lesions of the central nervous system or on the loss of rigidity of the bones at the epiphyseo-diaphyseal junction. The changes produced in the bones are very little different from the rickets which results from feeding rats on diets in which the phosphorus content is very low and the content of calcium is above the optimal for the growth of the animal. The bones of the animals which were fed on strontium have a deeper metaphysis, but not so great enlargement laterally of the ends of the long bones. The condition resulting from feeding the strontium-containing ration is in effect an exaggerated form of rickets.

The bones of Lehnerdt's animals showed a different condition, in which the epiphyseo-diaphyseal junctions were normal and the marrow cavity was almost or quite obliterated by numbers of small osteoid trabeculae. This condition,—"strontium sclerosis"—he believed to be closely related to the sclerosis of the bone, which Wegner described as resulting from administration of large amounts of phosphorus. We have been able to reproduce a similar picture by feeding animals on diets with a very high phosphorus content and a low content of calcium. Reference to Lehnerdt's diet will show that the basal ration (muscle meat and fat) was very low in calcium, but contained a very large amount of phosphorus. Moreover, the strontium added to his diet was given as $\text{Sr}_2(\text{PO}_4)_3$. There can be no doubt that the effect of feeding strontium is influenced by the ratio between strontium and phosphorus in the diet. The same is true in regard to calcium. It is evidently possible, by varying the ratio between phosphorus and either of the two alkaline earths, to produce either a condition which cannot be differentiated from rickets, or an osteosclerosis. The sclerosis obtained by Lehnerdt caused by strontium resembles the sclerosis which follows high phosphorus feeding, because it is the same condition. Sclerosis is not caused by strontium, phosphorus or calcium, but by a salt mixture in which the Ca ion is low as compared with the phosphate ion. In so far the behavior of calcium and strontium are alike, but although strontium is stored in the bone it cannot be made to serve the purpose of the lime salt. Thus, the administration of cod-liver oil to animals whose calcium has been replaced by strontium does not cause any change in the pathological picture produced.

CONCLUSIONS

1. When strontium replaces calcium in an otherwise satisfactory diet (2.2 per cent of strontium carbonate), it stimulates growth, and causes the bones to develop the characteristic picture of rickets "strontium rickets."

2. The anatomical lesions, described by Lehnerdt as induced by the administration of strontium to dogs, were not due to the strontium alone, but to a diet very poor in calcium and high in phosphorus, and deficient in the factors fat-soluble A or a second dietary essential which we have shown to be associated with certain fats, notably cod-liver oil.

3. Cod-liver oil does not enable the animal to compensate for a faulty diet containing strontium.

LEGENDS TO CHARTS

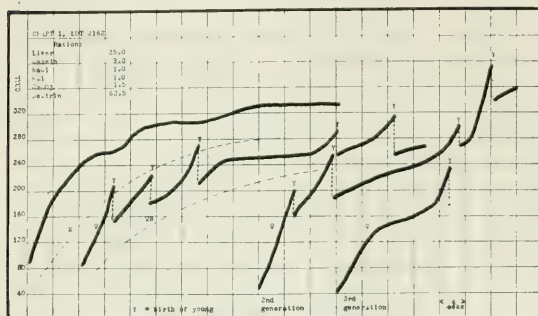


Chart 1. The records of the animals whose curves are shown illustrate the adequacy of the organic components of our diet 2162, which served as the food mixture in our experiments on the effect of strontium feeding on the histological structure of the skeleton. When calcium was added to this mixture, as in diet 2162, to the extent of 1.5 per cent, optimal growth and very good nutrition were secured over a long period. The 25 per cent of liver furnished all the fat-soluble A and water-soluble B which the animals required both for growth and repeated reproduction. Whatever content of the calcium depositing factor it contained was likewise derived from the liver. Their skeletons were of good quality, and were histologically normal. Growth curves for three generations are shown in the chart. This food mixture without the calcium addition is incapable of inducing any appreciable amount of growth (see Chart 2, Lot 2581).

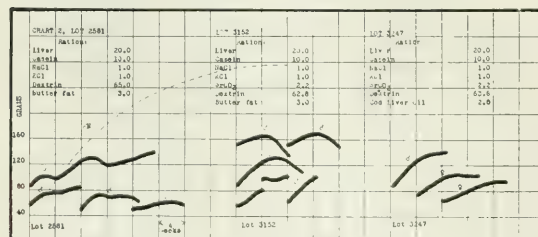


Chart 2. Lot 2581 had the same diet as that of Lot 2162 except that calcium was omitted. Three per cent of butter fat was added in its place. This diet was of very high quality except for its deficiency in calcium. A suitable addition of calcium converts it into a food mixture capable of inducing excellent growth and of maintaining a family of rats in good nutritive condition through at least three successive generations. These rats did not develop typical rickets, but the histological picture presented by their bones bore a fundamental resemblance to this disease. The description of sections of their bones will be found elsewhere in this paper.

Lots 3152 and 3247 had a diet like that of Lot 2581 except that strontium carbonate was added in amount sufficient to give the atomic equivalent of strontium for the calcium in 1.5 per cent of calcium carbonate. Lot 3152 received 3 per cent of butter fat and Lot 3247 received 2 per cent of cod-liver oil. The animals in both groups developed typical rickets.

It is of great interest that strontium stimulated osteoid production to such a marked degree. It led to the formation of the typical rachitic metaphysis, notwithstanding the presence of a great abundance of the substance furnished by cod-liver oil. This substance in the presence of strontium, under the defective nutritive conditions which are maintained by the diet administered to Lot 2581, failed to protect the animals against the pharmacological effects of strontium. It is further of interest to note that under the experimental conditions here described, cod-liver oil is no more effective than is butter fat in exerting a directive influence on bone development.

EXPLANATION OF PLATES

Fig. 1. Photomicrograph of the lower end of the femur of a rat, to show the lesion which follows the substitution of strontium for calcium in the diet. Note the wide metaphysis and the irregularity of the epiphyseal cartilage. The line formed by the rests of old calcified intracellular substance (r) across the bone at the junction of the shaft with the metaphysis marks the situation of the growing area where the effect of the strontium first became apparent. Note also the overgrowth of osteoid tissues, the osteoporosis of the shaft, and the normal medullary cavity.

Leitz micro-summer objective, 35 mm.—no ocular.

Fig. 2. To show the degeneration of the cartilage in the neighborhood of the blood vessels.

Leitz No. 6 dry objective—no ocular.

Fig. 3. Same magnification as in the preceding picture. The picture shows proliferation of the cartilage cells with resulting reduction in the size of the individual cells. From an area in which cartilage cells were undergoing metaplasia under the influence of the blood vascular system.

Fig. 4. High power photograph showing the osteoid tissue. Note the small size of the nuclei of the osteoid corpuscles and the lamellar arrangement of the osteoid.

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FIG. 1.

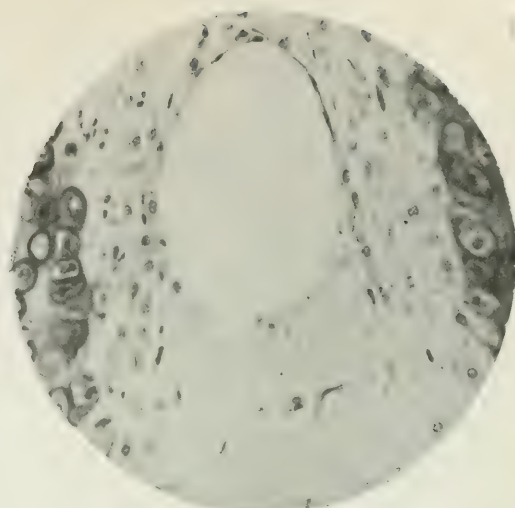


FIG. 2.

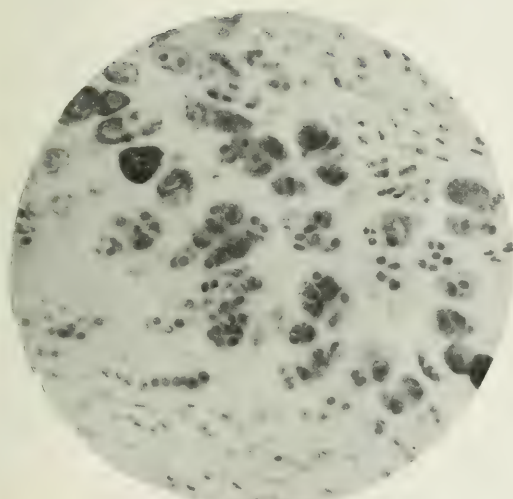


FIG. 3.



FIG. 4.

CARCINOMA OF THE CERVIX UTERI*

A VERY EARLY CASE

By KARL H. MARTZLOFF

(From the Department of Gynecology of The Johns Hopkins Hospital and University)

The case reported is one of very early carcinoma of the cervix. A panhysterectomy was performed and today the patient feels perfectly well. Of the ultimate result, however, nothing can as yet be said with certainty.

The patient, Mrs. M. M. (Gyn. No. 27080-27165) is a white woman, widowed, 55 years of age, who has had two normal pregnancies and two miscarriages. Her menopause occurred five years ago.

Three months before coming to The Johns Hopkins Hospital the patient began to have a profuse, watery, vaginal discharge which soon became blood-tinged and malodorous. On admission to the hospital, August 13, 1921, examination revealed nothing grossly abnormal. Nevertheless, the history of a foul-smelling, blood-tinged, watery, vaginal discharge commencing five years after the menopause was so suggestive of malignancy that it was decided to perform a diagnostic dilatation and curettage.

When the cervix was dilated, a small quantity of purulent material escaped, evidently from the uterine cavity. Curettement brought away a very small quantity of tissue, and not the large amount usually obtained in definite cervical cancer. Frozen sections made at the time of this operation showed nothing that could positively be identified as cancer, so the patient was returned to the ward. Celloidin sections made on the following day, however, showed tissue which we considered undoubtedly malignant, though by no means giving the picture of a fullblown cancer (Figs. 1 and 2).

The patient at that time refused a radical operation, and left the hospital, only to return in two weeks, when a modified Wertheim operation was done. Her convalescence was uneventful and she is now apparently well.

On macroscopic examination, the pathological specimen with its apparently normal cervix was, to say the least, depressing, for we felt that through a grave error

* Read before the Medical Society of The Johns Hopkins Hospital, November 7, 1921.

† This photograph was retouched in order to bring out more distinctly the smooth surface of the mucosa lining the vaginal cuff and cervix.

‡ In this connection it is only fair to state that when the sections represented in Figs. 1 and 6 were submitted to four well known pathologists, three considered the tissue to be cancerous and one did not consider the evidence sufficient to warrant such a diagnosis. However, after seeing the serial sections represented in Fig. 9, the opinion in favor of cancer was unanimous.

in diagnosis on our part the patient had been subjected to a serious and unnecessary operation. Figure 3,† is a photograph of the specimen in longitudinal section and tells graphically why, so far as could be seen, we were in the face of a surgical success but a diagnostic failure.

Pathological Examination: (Gyn. Path. No. 27099).—The specimen consists of the entire uterus with its adnexa and a liberal margin of vaginal mucosa. The uterus measures 7 x 4 x 3.5 cm. The vaginal cuff measures from 1 to 1.5 cm. in length and is normal in appearance. The lips of the cervix and the cervical canal are pale grey, smooth and glistening, and present nothing noteworthy. The uterine cavity is dilated, its walls are somewhat irregular, of a dull, dirty, grey color, and scraping brings away nothing in the nature of an exudate or a necrotic membrane. Blocks are taken from all portions of the cervix, uterus, parametrium, and adnexa.

Sections of the uterus show the myometrium infiltrated with numerous round and occasional polymorphonuclear leucocytes. No endometrium is present. The only other sections of interest are those taken from the area indicated at "A" (Fig. 3) on the anterior cervical wall. Here the cervical epithelium proximal to "A" has for the most part its usual appearance, but in certain areas it shows a definite departure from the normal. In Figure 4 both the normal and abnormal stratified cervical epithelium is seen. At *x* the epithelium is hyperplastic and shows a well defined metaplasia in which the epidermal cells have lost the normal gradation from stratum mucosum to stratum germinativum. Instead, one sees large oval and broad spindle-shaped cells rather uniform in size, with hyperchromatic nuclei which show some irregularity in staining reaction. There are some nucleoli, and mitotic figures (Fig. 5, *a*) are not uncommon. These cells have practically no eosin-staining cytoplasm.

Just distal to "A" (Fig. 3.) sections show cell-nests (Fig. 6.) in which the cells resemble those just described, although here the irregularity in size, shape, and staining reaction is more marked and the mitotic figures are more numerous (Figs. 7 and 8.).

We believe that this is an early carcinoma of the cervix uteri of a type which we have been seeing rather frequently of late and concerning the nature of which competent pathologists are not in accord.‡

Because of the difference of opinion which occurred after the reading of this paper, we were glad to follow

Dr. MacCallum's suggestion and make serial sections of the entire cervix, in order to find, if possible, some areas of more advanced carcinomatous invasion. Serial sections made from the right half of the cervix, from which the original blocks were taken, show a condition quite similar to that which has just been described. Serial sections from the left half of the cervix show cell-nests (Figs. 9, *b* and 10) similar to those represented in Figure 6, but in addition there are areas of actual lymphatic invasion (Figs. 9, *c* and 11), small clumps of cancer cells lying free in what appear to be endothelial-lined spaces. The microscopic invasion in none of these sections goes beyond a depth of 2 mm. below the surface of the cervical canal and the entire neoplastic process is limited to the anterior portion of the cervix. This, we feel, proves conclusively that our case is one of a true malignant neoplastic process and also demonstrates the value of making serial sections, in disputable cases, when numerous routine sections do not furnish the information desired.

DISCUSSION

Dr. Thomas S. Cullen: "Dr. Martzloff's squamous-cell carcinoma is evidently a very early one. We have never found nuclear figures in the squamous epithelium of the cervix except in cases of carcinoma of the cervix."

After discussing briefly an early squamous-cell carcinoma of the cervix, which he had reported in *Surgery, Gynecology and Obstetrics*, in August, 1921, Dr. Cullen added: "We fully realize that nuclear figures do occur in the squamous epithelium, otherwise there could be no reparation when defects occur. But they are so uncommon that we have not encountered them in our routine work except where malignancy exists. We have also failed to note them in the cylindrical epithelium of the cervix."

"On the other hand, nuclear figures are regularly met with in the epithelium of the body of the uterus, and in cases of hyperplasia of the endometrium nuclear figures are frequently found in the stroma cells of the mucosa."

THE CAPILLARIES OF THE BONE MARROW OF THE ADULT PIGEON

By CHARLES A. DOAN

(From the Anatomical Laboratory, Johns Hopkins University)

Since the time when it first became known that the red marrow of the long bones gives rise to certain of the essential cells of the circulating blood in vertebrates, the question as to the exact derivation and relationship of the different types of blood-cells to each other has proved most puzzling and fascinating to scientific investigators. To the clinician, also, the question has been, and is today, a most urgent one, upon the understanding of which will depend the treatment of certain well-recognized but as yet little-understood diseases.

This eagerness to understand the origin and development of the blood elements *per se* has relegated the question of the circulation and exact vascular pattern of the marrow to a position of minor significance, though its importance, at least for the delivery of these cellular elements, once formed, to the organism as a whole, is quite apparent. With but few exceptions, the majority of workers in the field have confined their efforts to the main issue of cellular derivation and development and have either ignored altogether or considered only incidentally and superficially the vascularization of bone marrow.

Three theories as to the nature of the circulation in adult marrow have been advanced, no one of which, however, has been established convincingly enough to be generally accepted, though all three have received more or less support. Rindfleisch¹ represents one extreme. He believed that his experiments showed that the blood

spaces are lined by parenchyma alone and have no endothelial walls. Langer,² on the other hand, believed the marrow to be supplied by an entirely closed vascular system. Bunting³ hypothesized vessels lined with endothelium but with openings at various points communicating directly with the medullary parenchyma. Drinker⁴ tended to support this view, though he expressed some doubt of the conformity of normal adult marrow to this pattern.

The greatest progress made toward solving the problem of the origin and evolution of individual types of blood-cells has been through embryological studies. Probably the most representative work on the embryology of the blood is that carried out by Danckhoff^{5, 6} and Sabin^{7, 8} on birds and by Maximow^{9, 10} on mammals. Both Maximow and Danckhoff recognized the relationship between endothelium and blood-cells, not only in the stage of the primitive blood-islands but also in somewhat later stages; both have thought, however, that endothelium gives rise only to indifferent blood-cells. Schridde,¹¹ on the other hand, has described the direct transformation of endothelium into erythroblasts in early human embryos. Maximow believed that although the early erythroblasts of mammalian embryos are intravascular in origin and derived indirectly from endothelium, the ultimate erythroblasts are a group of cells extra-vascular in origin. This question has now, however, been reopened by the work of Sabin,⁷ and it was not until she had

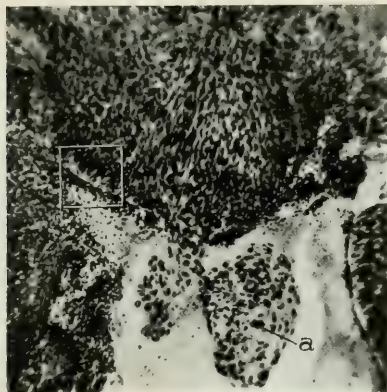


FIG. I.

FIG. 1.—(Gyn. Path. No. 27069.) Section of curetting showing the hyperplasia and metaplasia. At *a* is a multinuclear giant cell. There are also mitotic figures, see Fig. 2.

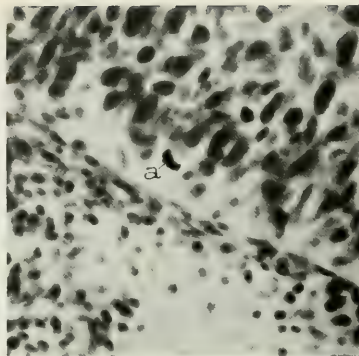


FIG. II.

FIG. 2.—(Gyn. Path. No. 27069.) A high power picture of the blocked area in Fig. 1. At *a* is a well defined mitotic figure in its metaphase. The irregularity in size, shape and staining reaction of the cells is also well seen.



FIG. III.

FIG. 3.—(Gyn. Path. No. 27099.) Showing smooth cervical lips and vaginal mucosa. The uterine cavity is dilated and was filled with purulent material. At "A" is the situation of the carcinoma which is not visible on macroscopic examination.



FIG. IV.

FIG. 4.—(Gyn. Path. No. 27099.) Low power photomicrograph from (Fig. 3) "A." At *x* is seen the epithelial hyperplasia which even under the low power reveals a metaplasia. Here the cells have lost the normal transition stages of the gradation from stratum mucosum to stratum germinativum.

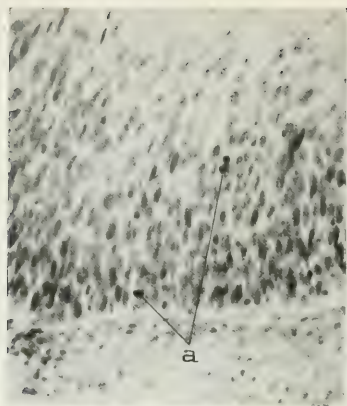


FIG. V.

FIG. 5.—(Gyn. Path. No. 27099.) A high power picture of the area enclosed in square in Fig. 4. At *a* are two mitotic figures, one in its metaphase and the other in its anaphase.

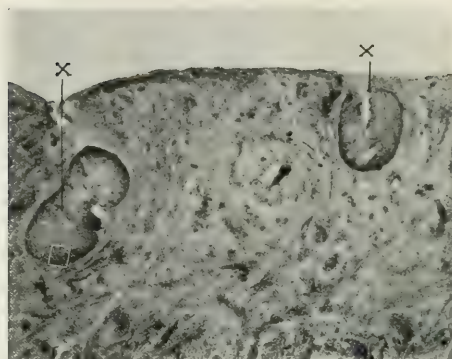


FIG. VI.

FIG. 6.—(Gyn. Path. No. 27099.) Low power photomicrograph of area just distal to "A" in Figure 3. The areas at *x* show typical carcinoma, the innermost nest of cells lying 1 mm. beneath the surface of the cervical canal.

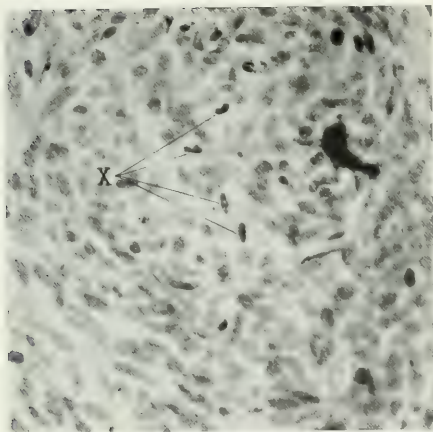


FIG. VII.

FIG. 7.—(Gyn. Path. No. 27099.) High power picture of area enclosed in square in Fig. 6. The mitotic figures are well seen at *x*.

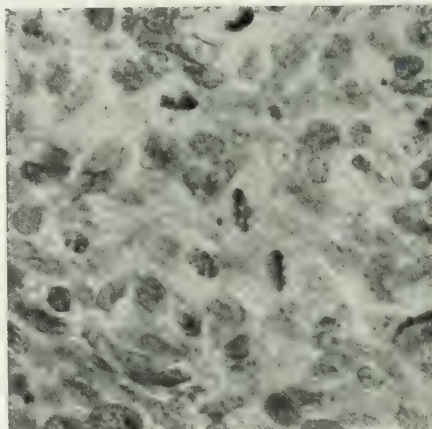


FIG. VIII.

FIG. 8.—(Gyn. Path. No. 27099.) A higher power picture of Fig. 7 area *x*, showing the metaphase stage of mitosis in three of the cells.

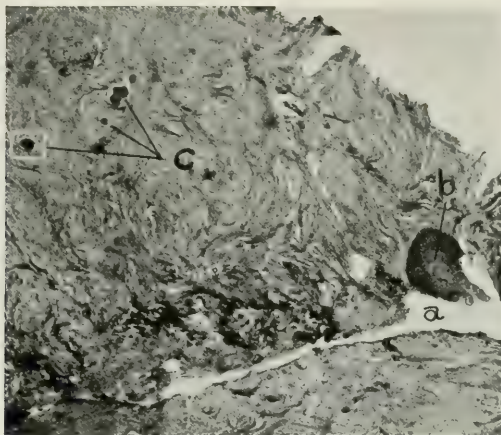


FIG. IX.

FIG. 9.—(Gyn. Path. No. 27099, Serial No. 60.) A low power picture of a section from left half of cervix. *a* shows cervical canal, *b* is a cell nest. *c* points to three cell nests in the anterior lip of cervix, 2 mm. from surface of the cervical canal.

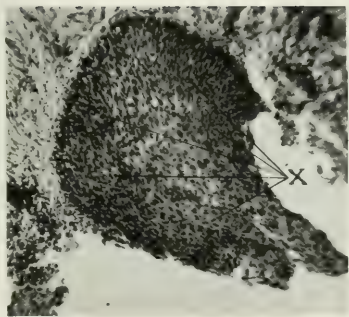


FIG. X.

FIG. 10.—(Gyn. Path. No. 27099.) A high power of *b* Figure 9. Numerous mitotic figures are seen at *x*. Under higher magnification the appearance is practically identical with that seen in Figures 7 and 8.

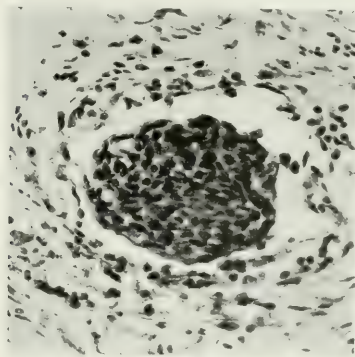


FIG. XI.

FIG. 11.—(Gyn. Path. No. 27099.) A high power picture of area inclosed in square in Figure 9.

actually seen, by direct observation on living chick embryos during the second day of incubation, the differentiation of the red cell from early endothelium, and later⁸ the origin of the monocyte cell-series and clasmacytes from the same source in chicks on the third and fourth days, that the etiological importance of the endothelium and hence the significance of the exact pattern of the vessels of the marrow of the mature organism was adequately emphasized. Stimulated by this work and its potential relationship to the production of blood elements in the functioning adult organism, certain questions of interest naturally arose. What is the extent and distribution of the endothelium in the adult marrow? Is the vascular system a closed one? Are the connecting vessels between the arteries and veins large sinusoidal spaces leading directly from arterioles to venules, or is there a capillary bed? If there is a definite capillary bed, how extensive is it and how distributed? Are all of the channels patent at all times or, in emergencies, is there an increase in the vascular bed? As already indicated, the information, concerning these points, to be obtained from the literature is quite unsatisfactory. More definite answers to these questions and a more thorough understanding of the architecture of bone marrow attendant upon them should afford the rational basis upon which to proceed with a further analysis of the relationship of the blood-elements to the endothelium in marrow.

This problem was begun at the suggestion of Dr. F. R. Sabin, and it is a pleasure to acknowledge here her continued interest and assistance throughout its development.

MATERIAL AND METHOD

The conclusions reached in this paper are based largely on a series of investigations on about forty adult pigeons. Further experiments of a similar character conducted on the cat, rabbit, and white rat seem to substantiate and corroborate the gross findings in the pigeon, though a larger series will be necessary for complete corroboration in the mammal.

The attempt was made to get complete injections of the vascular system of the bone marrow; india ink, one part, in physiological saline, three parts, proved to be the best medium for injections. My most successful injections were obtained with a pressure of 130 mm. of mercury. When the pressure was materially increased above this point, rupture and extravasation frequently occurred. With pressures below this point incomplete injections resulted.

The radius and ulna of one side were fixed and cleared by the Spalteholz¹² method, with the marrow *in situ*. The "marrow pencils" were removed from the bones of the opposite side, fixed in Helly's fluid at 38°C, dehydrated, cleared and embedded for sectioning, celloidin embedding proving better for the study of the individual cells, as pointed out by Maximow and Danchakoff. The

humerus in the pigeon has no bone marrow, in fact it is a pneumatic bone.

OBSERVATIONS

In the earlier incomplete injections the gross architecture of the bone marrow is plainly evident in the cleared specimens. The medullary artery enters the marrow cavity near the center of the diaphysis, perforating the compact tissue obliquely, and immediately divides into the main branches which diverge abruptly, one extending toward each epiphysis. These two main arterial trunks divide in turn about half way to the epiphyses and extend from the point of their origin to the end of the marrow.

In addition to this main arterial supply, there are to be seen numerous small vessels entering along the shaft of the bone, primarily to nourish the cancellous and compact tissues, but anastomosing at the periphery with the arterioles of the central vessels. Near the epiphyses, one or two small arteries are usually seen entering the marrow cavity through the bone. There is frequent and intimate intercommunication along the entire shaft between the nutrient vessels of the Haversian canals and the circumferential end-arterioles and venules of the medulla of the bone. These anastomoses form a very striking picture in a cleared specimen and give a new insight into the delicacy of the vascular interlacings and the extent of their ramifications. We are not dealing with two more or less separate and distinct systems, one to nourish the marrow and the other cancellous and compact tissues, but with one interdependent and communicating system.

There are three groups of veins in the long bone: (1) From one to four parallel veins accompany the artery and traverse the shaft from each end, to unite near the center in a single efferent vein. Thus there seems to be a group of main central vessels which arise from the nutrient artery and corresponding vein and extend the full length of the bone from a zone near the proximal almost to the distal epiphyseal line. (2) Several large veins emerge near the articular extremities from the extensive vascular area of red marrow, always more prominent toward the epiphyses. (3) There are numerous small veins along the diaphysis, which drain the compact tissue and the peripheral area of the marrow, and, with the small nutrient arterioles of the shaft, form the abundant vascular network of the periosteum.

This general vascular pattern holds for both the radius and ulna of the pigeon, the individual bones differing only in the number of their central vessels in direct relation to their relative size and the extent of bone marrow to be vascularized. In relatively complete injections, the central vessels cannot be seen from the surface in even the most perfectly cleared specimens, so dense is the network of injected vessels, as will be indicated later.

Specimens showing the next stage of a partially complete injection illustrate again the gross picture observed in lesser injections of the marrow cleared *in situ*. The central vessels are still visible and it may be seen that there are smaller branches coming off at an angle from the main arteries, which extend toward the circumference. These begin almost at the center of the shaft but become more numerous and dense toward the ends. At each epiphysis there is a veritable spray-like shower of fine vessels which ramify to every part of the marrow, and supply the epiphyses as well, but stop abruptly at the line of cartilage which forms the articulation of the joint. The characteristic vessels of embryonic cartilage have disappeared in the mature state.

The artery and its branches are easily distinguishable from the veins by virtue of their smaller calibre, firmer wall, and less tortuous course, and also by the fact that the lumen is more closely packed with the particles of carbon. The divisions of the artery are characteristic; the branches come off at an acute angle and the subdivisions are much less numerous than those of the corresponding veins. The arterioles at the periphery are characteristic in their delicacy, scarcity, and apparently limited distribution.

The venous system presents a similar picture though greatly magnified and multiplied. Coming off from the central vessel, almost at right angles, are the large distended veins which at once branch outward toward the circumference in an ever-widening balloon-shaped bed to anastomose eventually with branches from tufts on either side. The large calibre of the vessels is strikingly maintained and though there is some decrease in the lumen toward the periphery it is not commensurate with the extent of the branching. The most apparent and striking thing about the entire vascular system of the bone marrow, both in gross and in microscopic view, is this extensive venous ramification and its very evident capacity for large quantities of blood.

A still better comprehension of these venous and arterial tufts and the means by which they become continuous with each other is obtained from a more complete injection, in sections 100 to 150 microns thick. In such preparations can be plainly seen what I have termed the "transition capillaries" leading directly from the arterioles to the venous sinusoids and apparently with very little true arterial capillary bed. This patent capillary bed connecting arteries and venules is extremely circumscribed and it is not until the venous sinusoidal anastomoses are reached that the blood spreads out in lacing and interlacing vessel tufts, thence to be directed from the tuft-like branchings into larger and larger vessels, eventually to enter the central longitudinal vein almost at right angles, or to find egress by way of one of the other venous outlets. The marrow appears to be divided almost into segments or lobules by these venous

tufts, so completely do they ramify in definite areas, anastomosing on all sides with the ramification of bordering tufts. The relationship of the arterial tree to the venous tufts on either side and the capillary transitions from one to the other, even though not extensive, are easily distinguishable and very characteristic. There is little doubt that these extensively distributed, spacious, thin-walled venous sinusoids form normally the real functioning vascular bed of the marrow. These are the vessels which have been seen and described as the fundamental units of the bone marrow by those who have written in this field. By most workers they are termed the *venous capillaries*. It would seem that *venous sinus* or *venous sinusoid* might be the more appropriate and desirable terminology, inasmuch as there are already two types of true capillaries in the marrow as recognized and interpreted in these observations.

All of the vessels thus far described are plainly apparent, either grossly or with the aid of the binocular microscope. The analysis of the circulation up to this point has been comparatively simple, but it was when an attempt was made to study under the oil immersion, the detailed ramification of the smaller vessels, the extent and continuity of the endothelium, that difficulties were encountered. It was soon found that analysis of these finer points in a normal marrow would be very unsatisfactory and impracticable. In order to analyze with any certainty the finer ramifications of the vascular pattern, it is essential in the first instance, at least, to have a marrow depleted, as far as possible, of all the free cells. An attempt was therefore made to produce experimentally a hypoplastic bone marrow in the pigeon. The desired condition was secured through simple starvation for a period varying from ten to eighteen days.

In such a hypoplastic marrow three types of cells were observed—fat cells, reticular cells, and endothelial cells. In order to analyze the relations of these three cell types the vessels of the marrow were washed out with salt solution and then injected with India ink. The fat cells, together with their nuclei, were readily distinguishable and quite characteristic. They were more numerous in the hypoplastic marrow, having replaced apparently to a large extent the depleted cellular areas. In the fixed tissue these cells appear as empty spaces, limited by a thin but distinct membrane; and each contains a more or less flattened oval nucleus, eccentrically placed, and but faintly stained, owing to the small amount of chromatin. Such cells make an easily discernible network. Frozen sections of the fresh tissue, stained with Sudan III, indicate the increased extent of these deposits of fat in the depleted marrow.

Reticular elements which conformed to all of the known criteria were to be seen. They were large pentagonal or hexagonal cells with large, round vesicular

nuclei; the cytoplasm took a faint eosin stain, the nuclei showed a moderate content of chromatin.

The endothelial cells in the main conformed to certain standards and were recognized through various characteristics. In the areas where the endothelium could be seen lining the venules and the capillaries connecting them, there was no difficulty in its identification, but there are capillaries in the bone marrow where, even after taking all the histological characteristics of endothelium into consideration, the cells can not be definitely classified in normal uninjected marrow. Unfortunately, a specific stain for endothelium has not been developed up to the present time and such characteristics as size, morphology, and peculiarities of the nuclei are not adequate criteria. Realizing fully, then, the difficulties of final determination in the case of a certain few individual cells, I have tried to analyze the picture presented by these injections on the basis of the data available at the present time for their interpretation, while recognizing the limitations of our methods.

It is not until sections as thin as 5μ (Figs. 1 and 2) from a relatively complete injection of a hypoplastic marrow, are seen under the oil-immersion that the full import of the nature and extent of the bone marrow vascularization begins to be realized and understood. First of all, the gross structures—the main longitudinal vessels, transverse smaller branches, arterioles, a few transition capillaries, and venous sinusoids described above—are easily verified in the serial sections. But in addition to these, I have found, appearing between the fat spaces in well-outlined and clearly defined channels, a most extensive system of capillaries, hitherto unsuspected. Many of these capillaries appear to have been non-patent and functionally dormant so far as the circulation is concerned. They are collapsed so that only a trace of fine ink granules reveals the presence of a potential lumen, the calibre of which appears insufficient for the passage of even a single blood-cell element without difficulty. In an ordinary injection they are totally collapsed and are seen as septa surrounding the fat cell spaces. Toward the epiphyses there is a complete encircling of each fat space by these channels. They are seen to lead directly from the large venous sinusoids via typical conical openings and appear to be continuous with them. There is no break in the continuity of the endothelium which forms these slender channels from sinusoid to sinusoid. There is no extravasation at any point and the material injected follows these vessels everywhere. It is evident that these channels are closed in the sense that there is no extravasation or diffuse permeation of the tissue by the injected ink.

The attempt to differentiate an extravasation from a true circumscribed distribution of perfused particles within definite channels was not made without a full

appreciation of the marked tendency of such granules to follow a reticular framework closely in any injection into diffuse connective tissue. This characteristic of reticular tissues to be outlined by extravasated particles, thus simulating, more or less, definite channels, is recognized and acknowledged, and it is obvious that the possibility of error of interpretation in injections of mesenchymatous tissue requires a corresponding amount of attention and care in analysis.

There are, however, five points apparent in the interpretation of these studies which emphasize strongly the non-fenestrated character of the vascular bed of the bone marrow: (1) In injections showing a diffuse permeation of the medullary parenchyma there have been demonstrable ruptures in vessel walls. (2) In extravasation it is clear that the extruded granules are neither phagocytized nor regularly distributed along one side, but adhere promiscuously and heterogeneously to the surface of the parenchymal cells, thus more or less concealing their outline. In contrast to this, the particles within a definite lumen are scattered here and there along the sides of the lining cells on the inside of the channel only. (3) In an analysis of comparatively complete injections, showing this extensive, intersinusoidal capillary bed, not only can these channels be distinctly followed by the granules of ink, but the reticular network or framework of the medullary parenchyma can be seen in the same areas without any granules of ink attached thereto. (4) The walls of the veins and venules appear as continuous endothelial-lined channels, similar in appearance to the vascular bed elsewhere in the body, but with conical openings into the tiny capillary network. (5) Finally, I have gotten relatively complete injections of these very fine, extensive, lace-like vessels without the slightest evidence of any of the injected particles outside the closed channels in the parenchyma. In other words, there is no evidence of any fenestrated vessel wall, similar to that described by Mollier¹³ for the spleen, in the adult bone marrow studied here. One only need contrast a true extravasation with one of these injections to recognize the difference at once. It is, however, very possible that, in an injection of normal bone marrow which filled only arterioles, transition capillaries and venous sinusoids, these conical capillary openings might be interpreted as fenestrated membranes.

The endothelial cells are thinned out, in contrast to their number and arrangement in a larger vessel, and in many instances have been forced apparently into the interstices between encroaching fat cells and look more nearly like the primitive embryonic endothelium. They can, nevertheless, be seen to line these spaces through which granules of the injected fluid have been forced. The picture, then, is that of a very extensive capillary bed which simulates in the appearance, distribution, and arrangement of its vessels and cell elements, an embry-

onic plexus rather than the ordinary mature capillary plexuses elsewhere recognized in the adult. This plexus is lined everywhere with endothelium.

SUMMARY

1. The arterial supply of bone marrow is secured via the medullary artery, the periosteal vessels along the shaft, and some vessels near the articular extremities which supply the epiphyses as well. The arterioles are relatively few in number.

2. Normally there are a few "transition capillaries" functioning as the intermediary communication between arterioles and venous sinusoids.

3. The very extensive distribution of large-lumened, thin-walled venous sinusoids, probably forming the real functioning vascular bed of the marrow, is the most characteristic thing about the gross circulation in bone marrow. The venous drainage is threefold, corresponding to that of the arterial supply.

4. A hypoplastic marrow is essential for the analysis of the finer distribution of the blood channels. In such a marrow can be seen a very extensive inter-sinusoidal capillary plexus, hitherto unsuspected, its normal state being possibly one of collapse.

5. The vascular system of the bone marrow is a closed system, no fenestrated vessel walls being demonstrable in this series of experiments.

6. Endothelium apparently forms a continuous lining throughout the vascular ramifications in the marrow, being therefore much more extensively distributed through the medium of the wide spread capillary plexus than has been indicated in the usual marrow injections heretofore described.

This report presents one of the active phases of the present day bone-marrow problem. The complete communication, with review of the literature, technic employed, and a series of plates illustrating the findings is being prepared for the Contributions to Embryology, Carnegie Institution of Washington.

FIGURE LEGENDS

Fig. 1.—Drawing of a hypoplastic bone marrow, injected with India ink, showing venous sinusoids and the inter-sinusoidal capillaries; from the radius of an adult pigeon (19A): *e.c.*, endothelial cells lining the capillaries; *r.c.*, reticular cells of the marrow; *n.f.c.*, nuclei of the fat cells; *r.b.c.*, red blood cell; *v.s.*, venous sinusoids; *cap.*, inter-sinusoidal capillaries surrounding the fat cells, with the granules of carbon of the injection fluid scattered throughout the extent of their chan-

nels. These capillaries are seen to be in direct communication with the large venous sinusoids. H. & E., 5 μ , x 700.

Fig. 2.—Photomicrograph of the same area in the bone marrow, the detail of which is given in Fig. 1. 5 μ , x 700.

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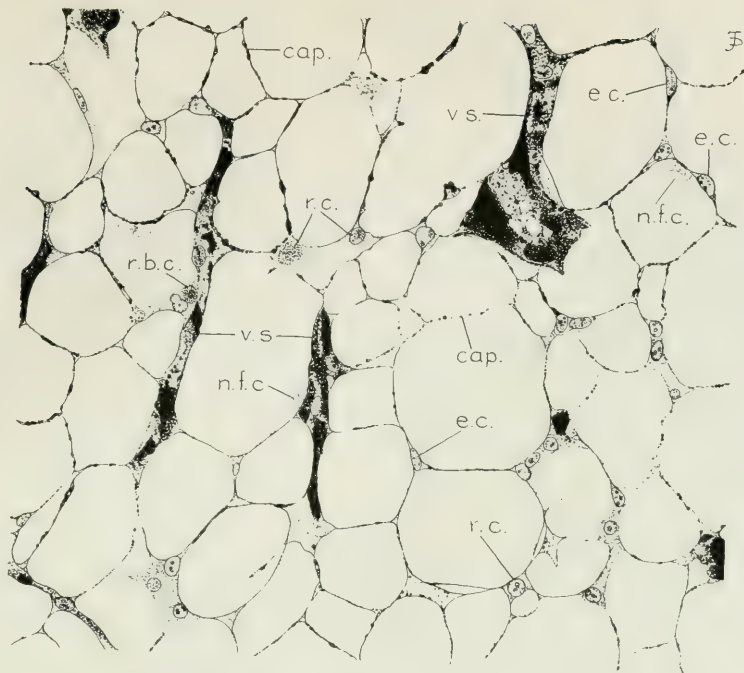


FIG. I.

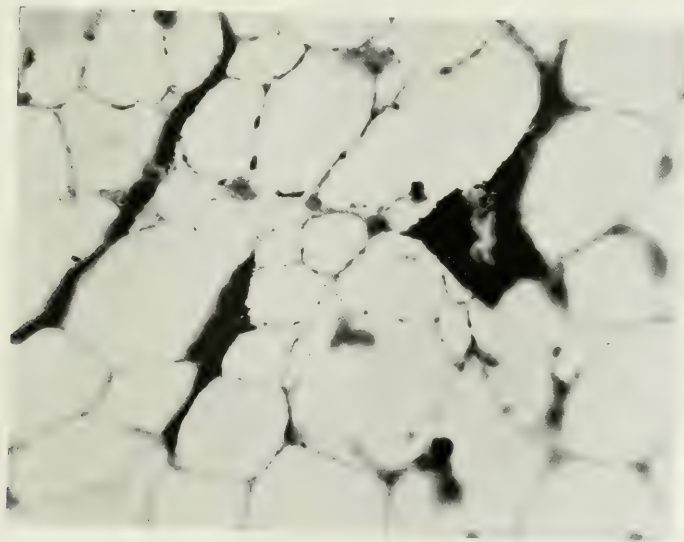


FIG. II.

THE EFFECT OF SLIGHT INCREASE OF TEMPERATURE ON THE BACTERIOSTATIC POWER OF GENTIAN VIOLET

By JOHN W. CHURCHMAN

(New York)

That bactericidal agents are more effective if applied hot than if applied cold is perfectly well known, although advantage is seldom taken of this knowledge in practice. From the experimental evidence herewith reported it would seem, however, that the degree to which bacteriostatic effects may be influenced by rises in temperature is not sufficiently appreciated.

The organism used in the experiments was peculiarly suited to the purpose, for it was an impeccable bacteriostatic strain, the descendant of a single bacterial cell planted on gentian violet agar.¹ This culture did not, therefore, contain the two types of organism (gentian violet positive and gentian violet negative) which I have shown may co-exist in at least some species of so-called "pure cultures" of *B. coli*.²

If gentian violet at room temperature was added to a suspension of this organism and the dye and bacteria allowed to remain in contact for one hour before planting the bacteria on plain agar, the dye was found to be without influence on the growth of the organisms (Fig. 1c). That is to say: this organism—and the same is true of about 90% of all Gram-negative bacteria—was gentian violet negative, as distinguished from the large majority of Gram-positive bacteria which do not survive exposure to the dye at room temperature, or even at ice temperature (e. g., *B. subtilis*, Fig. 1s).

If the temperature of the dye be slightly raised, however, a different story is told. Figs. 2 and 3, for example, represent experiments in which the strain of *B. coli* under examination has been exposed to stain which is kept at slightly elevated temperatures. At 35° C. the dye was without effect;³ organisms exposed for 1½ hours to dye kept at this temperature grew as well as if nothing had been done to them (Fig. II).

Heated to 50°, on the other hand, the dye became an efficient bactericide and organisms which had been exposed to gentian violet at this temperature refused to grow when planted on plain agar (Fig. III). That is to say: a bactericidal agent harmless for this strain of *B. coli*, when applied at room temperature, becomes potent if applied at 50°.

That neither of the two agents employed in this experiment—gentian violet and heat—is *alone* capable of bringing about the death of the organism—(capable of this, that is to say, in the degree used in the experiment)—is easily proven. Such an experiment is represented in Fig. IV. The plate marked A has been planted with four strokes of bacteria which have been exposed for one hour to gentian violet at room temperature; growth has been uninfluenced by the dye alone. The plate marked B has been inoculated with four strokes of an emulsion of the organism which has been kept at 50° C. for one hour; there has been no inhibition of the growth of the organisms due to the heat alone.

DEGREE OF HEAT NEEDED

No attempt has been made to determine the exact temperature at which gentian violet—a dye without effect on this strain of *B. coli*, if applied at room temperature—becomes bacteriostatic. Indeed, certain observations make it seem likely that the lethal dye-heat temperature is not a constant, but varies with the age of the organism used in the experiment and with other little understood factors. It does seem certain, however, that the increase in temperature necessary to effect this change is not very great. In one series of experiments, for instance, exposure to the dye at 30° for 1½ hrs. resulted in the death of nearly all the organisms, though exposure to the dye for the same length of time at room temperature (which, on the day of the experiment, was 26°) was without effect. A result of this kind, with so slight an increase of temperature, could not be constantly obtained; but the experiment indicates how slight an increase of temperature *may* be needed to make this impotent dye potent. 55° for 1 hr., or 50° for 1½ hrs. seems to be constantly bacteriostatic for this strain of *B. coli*—a result which should be compared with that of exposing a Gram-positive organism, like *B. subtilis* to gentian violet at ice temperature: for this Gram-positive organism the dye is bacteriostatic even at these very low temperatures.

EXPLANATION OF THE PHENOMENON

The reason why this slight degree of heat renders an impotent dye bacteriostatic is not altogether clear. It might seem that organisms which survived exposure to the unheated dye, but were killed by the dye when slightly heated, died—in the latter instance—because they were

¹ J. Exp. Med., 1921, XXXIII, 583.

² J. Exp. Med., 1921, XXXIII, 369.

³ This was the case in this particular experiment; as will later appear the results were not constant as to the exact temperature at which the dye became potent, and in some experiments even so slight a rise of temperature as this was sufficient to produce the effect.

subjected to two slight insults which (though neither alone was able to kill the bacteria) together were able to bring this result about. That this is not the case may be readily shown. If the organisms be subjected to the dye at room temperature and then to heat (50° for one hour), *in succession*, they grow as readily as if nothing had been done to them. That is to say: they must be exposed to the *heated dye* and not simply to *heat and dye* (Fig. V).

It is well known that the penetrative power of chemical agents is increased by heating them and it is possible that this may explain the potency of heated gentian violet for this strain of gentian-negative *B. coli*. No such increase in penetrative power is, however, demonstrable. In Fig. VI is shown a micro-photograph of organisms which have been stained by exposure to gentian violet for one hour at room temperature, and in Fig. VII organisms which have been exposed to the dye at 50°; if there is any difference in penetrative power of the dye as measured by depth of staining, it is in favor of the unheated dye, for the organisms which had been exposed to it are even darker than those stained with heated dye.

The parallelism between the Gram reaction and the gentian reaction, established in 1912,* might be invoked for light on the phenomenon under discussion. It is, in general, the Gram-negative organisms which survive exposure to gentian violet and the Gram-positive organisms which are killed by it. The strain of *B. coli* used in the experiments described, was, as already stated, an impeccable gentian-negative strain; it was definitely Gram-negative; and it survived exposure to gentian violet at room temperature, as the great majority of Gram-negative organisms do. It did *not* survive exposure to dye heated to 50°. Did this heating fix the dye so that it would be difficult to remove it with alcohol? And did this fixation account for the acquisition of potency by the dye? And do Gram-positive organisms owe their inability to withstand exposure to unheated dye to a similar power of fixation, which is—in their case—expressed by their Gram-positiveness? No such fixation of the dye by heating can be demonstrated; organisms which have been exposed to heated dye are quite as readily decolorized by alcohol—quite as definitely Gram-negative—as those which have been stained with unheated dye.

The difference in behavior of this strain of *B. coli* toward heated and unheated dye is at present, therefore, not to be explained. But the fact is established.

THERAPEUTIC SIGNIFICANCE

It is clear that if this dye possesses any therapeutic value when applied at room temperature one is justified in expecting its potency to be considerably increased by slight increases of temperature. *Not only should the dye be more toxic for Gram-positive organisms when applied hot than when applied cold; it should, when applied hot, become bacteriostatic for certain Gram-negative organisms on which, when applied cold, it is quite without effect.*

And what is true of this dye is probably also true of many other bactericidal agents. The fact should be borne in mind in the extensive studies of this subject now being carried on, in order to determine whether the value of the antiseptics under study may not be considerably increased in this simple way.

As regards gentian violet itself, it is clear that the technique described as having been used with a certain degree of success in the treatment of purulent arthritis *should be modified by the use of hot dye* (after lavage), instead of dye at room temperature.¹

And the same is true of the use of the dye, by the method of lavage and staining, in the treatment of empyema which will probably follow the work of Waters² and of Major,³ who have demonstrated that the method is feasible and have obtained suggestive results.

It is true that Gay and Morrison⁴ have published an experimental study of the treatment of empyema with aniline dyes which leads them to conclude that these substances have little or no future in this condition. But I am inclined to think their conclusions unnecessarily pessimistic. One would hardly expect that the instillation of dye or any other substance into a chest containing a septic effusion, without preliminary mechanical cleansing by aspiration and lavage, would accomplish much, if it accomplished anything. Yet in the majority of the experiments of Gay and Morrison this was the technique employed. Even so, they were able—in many instances—to bring a thorax which had been infected with streptococcus to complete, or almost complete, sterilization. Their conclusion that dyes would never be of value in the treatment of empyema was based on the fact that in these experiments if sterilization was achieved re-infection always occurred. This is exactly what one would have expected if the fact, to which I have so often called attention, were sufficiently appreciated: that these dyes are bacteriostats as well as bactericides. It is not to be expected that every organism, or indeed the majority of organisms, will be reached and killed in a cavity like the chest; but it is not impossible that, if a moderate amount of the dye be kept continually present, growth of organisms would be very difficult, if not impossible.

In attempting to sterilize the sinuses of the head with gentian violet, in human beings, I have had experiences

* J. Exp. Med., 1912, XVI, 221.

¹ J. A. M. A., 70: 1047 (Ap. 13) 1918; 72: 1280 (May 3) 1919; 74: 145 (Jan. 17) 1920; 75: 583 (Aug. 28) 1920; 77: 24 (July 2) 1921.

² Amer. Rev. Tuberculosis, Feb. 1921, No. 12, p. 875.

³ Amer. Jour. Med. Sc., CLXII, Sept. 21, No. 3, p. 397.

⁴ Journ. Inf. Dis., 1921, XXVIII, No. 1, p. 1.

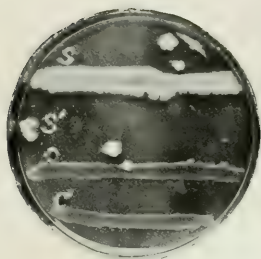


FIG. I.

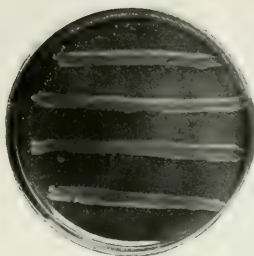


FIG. II.

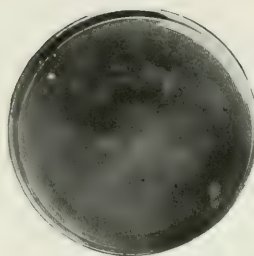
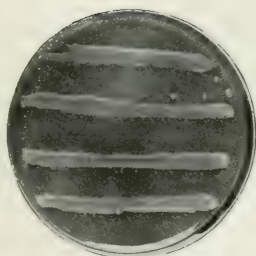
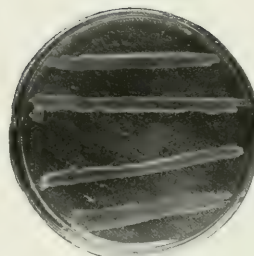


FIG. III.



A



B

FIG. IV.

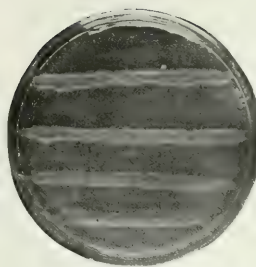


FIG. V.

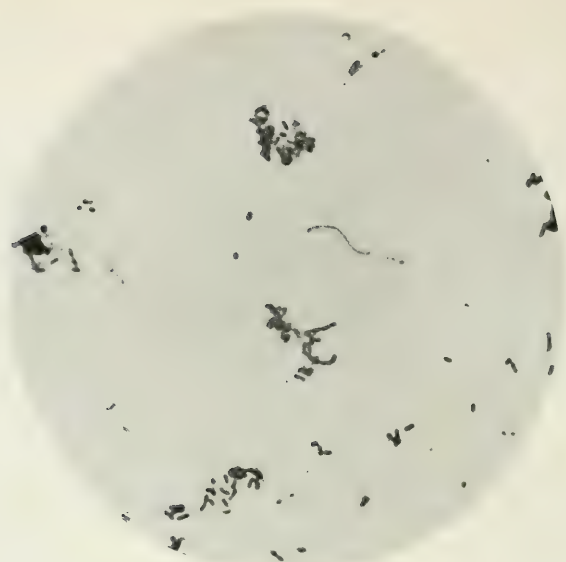


FIG. VI.

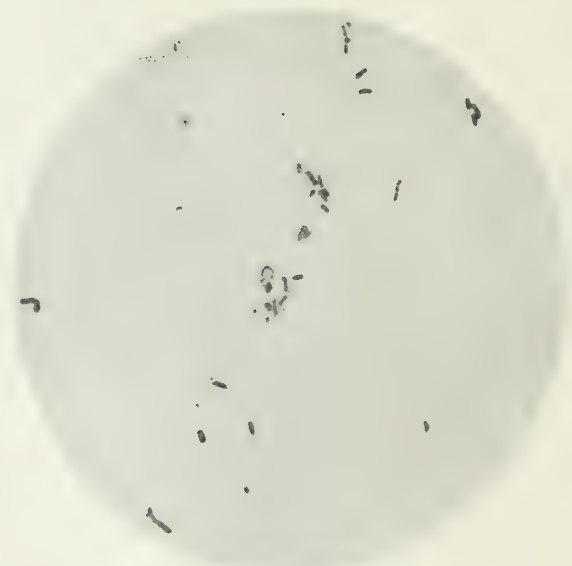


FIG. VII.

similar to those of Gay and Morrison. Even in cases of long standing infection negative cultures may be promptly obtained by mechanical cleansing and instillation of the dye; but re-infection always occurs.

But it is becoming more and more clear that substances which hold bacteria in check, even though they do not kill them, may have real therapeutic value. In localized infections a *sterilisans magna*, though ideal, is not essential. It is well known that in some instances, removal of the great mass of infection from a chest cavity by aspiration has been, alone, sufficient to enable the body to overcome the remaining infection; and it is not unreasonable to hope that if aspiration through a large needle be supplemented by lavage (as I have suggested) and by the instillation of a bacteriostatic agent like gentian violet, a fairly large percentage of these cases may be held in check until the body is able to overcome the infection. At least, the results already obtained with this method, incompletely applied, justify further study along the same lines. For this purpose, the stain should be used hot. It is of course, perfectly clear that dye instilled hot into the body cavities will not remain hot indefinitely, but will, relatively soon, cool to body temperature. We can expect however that during the time when this change in temperature is taking

place the potency of the bacteriostatic agent will be greater if the dye has been heated.

FIGURE LEGENDS

FIG. I.—To show that *B. subtilis* is killed by staining with gentian violet, *B. coli* unaffected. The organisms have been stroked, after being stained, on plain agar.

S = Unstained *subtilis*, control.

S' = Stained *subtilis*.

C = Unstained *B. coli*, control.

C' = Stained *B. coli*.

FIG. II.—*B. coli* stroked on plain agar after having been stained with gentian violet at 35°; growth unaffected. Cf. with Fig. III.

FIG. III.—4 strokes have been made of *B. coli* stained with gentian violet at 50°—no growth. Cf. with Fig. II.

FIG. IV.—Showing that neither heat (50°) alone, nor stain alone is sufficient to kill *B. coli*.

A—Stroked with organisms which have been heated for 1 hr. to 50°.

B—Stroked with organisms which have been stained for 1 hr. at room temperature.

FIG. V.—To show that exposure of *B. coli* to heat and to gentian violet in succession does not kill the organisms. Plate stroked with organisms which have been heated to 50° for 1 hr. and then stained for 1 hr., growth has been unaffected.

FIG. VI.—*B. coli* which have been stained with gentian violet at room temperature; penetration has been as deep as in those stained with dye at 50°. (See Fig. VII.)

FIG. VII.—*B. coli* which have been stained with gentian violet at 50°. Cf. Fig. VI.

STUDIES ON EXPERIMENTAL RICKETS. XXI.

AN EXPERIMENTAL DEMONSTRATION OF THE EXISTENCE OF A VITAMIN WHICH PROMOTES CALCIUM DEPOSITION

By E. V. MCCOLLUM, NINA SIMMONDS,

J. ERNESTINE BECKER

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore)

and

P. G. SHIPLEY

(From the Department of Pediatrics, The Johns Hopkins University, Baltimore)

Although there is no longer any room to doubt the efficacy of cod-liver oil in curing rickets, the nature of the active principle in the oil is still unknown. It has been stated that the fat-soluble A is responsible for the beneficial effect from its administration in this disease. We have, however, published certain evidence which led us to believe that the protection against rickets which cod-liver oil affords is not due to fat-soluble A.

Hopkins has shown that this vitamin is readily destroyed by oxidation. Following his lead we oxidized cod-liver oil for from 12 to 20 hours at 100° C by blowing air through it. Oil which was treated in this way did not cure xerophthalmia, even though its administration was begun at the onset of the disease, when edema of the eyelids was first noticeable. On the other hand, it was just as effective in curing rickets in rats as the untreated oil. The bones of rachitic animals which were given daily a

curative dose of the oxidized oil to the amount of 2 per cent of the weight of the ration (average daily consumption of cod-liver oil about 70 mgs.) for 11 days showed quite as advanced evidences of healing as those shown by animals which had received the same amount of unoxidized oil for the same length of time.

The anti-rachitic effect of cod-liver oil is not due, therefore, to its content of fat-soluble A, but to some other factor which the oil contains.

The existence of this substance, which exerts its effects in so remarkable a manner on the growing bones, is now as firmly established as is the existence of any of the three hitherto recognized vitamins. From its mode of action, and the very small amounts of it which are required to exert its directive influence on metabolism, it must, we believe, be classed with this group of essential nutritive principles.

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STUDIES IN ASYMPTOMATIC NEUROSYPHILIS

II. THE CLASSIFICATION, TREATMENT, AND PROGNOSIS OF EARLY ASYMPTOMATIC NEUROSYPHILIS *

By JOSEPH EARLE MOORE, M.D.

BALTIMORE

(From the Syphilis Department of the Medical Clinic,
The Johns Hopkins Hospital)

The work of many observers during recent years has demonstrated that in a large proportion of all patients who have contracted syphilis the central nervous system is invaded by the *Treponema pallidum* at the time of its general dissemination in the first months following infection. This invasion is easily detected, in most instances, by early examination of the cerebrospinal fluid. The percentage of patients thus found to have

* This clinical research has been aided by grants from the United States Interdepartmental Social Hygiene Board and from the American Social Hygiene Association.

early fluid abnormalities approximates the incidence of late clinical neurosyphilis—one fact which makes it probable that when central nervous system invasion does take place, it practically always occurs during the first months of the infection rather than at some later period. If this viewpoint be accepted, it is essential to examine the conditions under which neurologic invasion may occur. In addition, this paper will discuss the clinical and laboratory methods necessary for its detection, and the response to treatment of these early forms of neurosyphilis.

Leaving out of consideration the occasional precocious appearance of the characteristic late forms of clinical neurosyphilis such as paresis, tabes, etc., we have classified early neurosyphilis appearing within a year or less from the date of infection under five clinical groups:—

1. *Acute syphilitic meningitis*, occurring in previously untreated patients. It may be recognized as syphilitic by the accompanying signs of a recent early syphilis and by the characteristic cyto-biology of the cerebrospinal fluid.

2. *Precocious vascular neurosyphilis*, indicated by transitory aphasia, monoplegia, hemiplegia, etc.

3. *Neurorecurrence*. This type occurs only under definite conditions, *i.e.*, in a patient with early syphilis who has received an amount of treatment insufficient to eradicate the disease, which is then followed by a complete lapse of treatment. The clinical manifestations are varied, but usually consist of a sub-acute meningitis, commonly with, but sometimes without, focal cranial nerve lesions (most often paralyzes of the seventh or eighth cranial nerves). Occasionally epileptiform seizures occur.

4. *Neurosyphilis manifested by mild symptoms or slight physical signs, not of themselves diagnostic of central nervous system damage*. Patients in this group may complain of headache, neuralgic pains, insomnia, vertigo, or "nervousness," or may have no symptoms. Those with symptoms may or may not present the minor physical abnormalities which characterize the symptom free group, such as slight pupillary abnormalities (myosis, mydriasis, anisocoria, irregularity, or sluggish light reaction), and exaggeration, sluggishness, or inequalities of the reflexes. These symptoms and signs are not pathognomonic and may occur in non-syphilitic patients.

5. *Asymptomatic neurosyphilis*. The patients in this

group have no complaint, and show no physical abnormalities. The neurologic invasion is detected only by examination of the cerebrospinal fluid.

The first two of these five groups, precocious meningeal and vascular neurosyphilis, are rare, and for the purposes of this paper may be disregarded. The group of neurorecurrences has been comprehensively studied by others.¹

Groups 4 and 5 constitute the largest and most important class of patients. Owing to their minor character, the symptoms and signs detailed under the fourth group may be disregarded for the purpose of discussion, and these two groups considered together under the common name of early asymptomatic neurosyphilis. It will be shown that the recognition of these cases is easily accomplished; that from the ranks of these patients may arise certain of the future outspoken neurosyphilities;² that a study of the spinal fluid abnormalities observed permits a division into three sub-groups; and that the adjustment of treatment to the type of case will usually bring about a clinical and serologic "cure."

This paper is based on the study of 352 patients with primary or secondary syphilis from the Syphilis Department of the Johns Hopkins Hospital. In this series are arbitrarily included those patients whose disease on admission was of less than one year's duration. The available data in all cases consist of anamnesis, careful physical and neurologic examinations, accurate details of treatment, and blood and spinal fluid examinations. The last usually includes cell count, globulin test (Pandy), Wassermann test with 0.2, 0.4, and 1.0 cc. of fluid, with both plain alcoholic and cholesterinized antigens, and colloidal gold and mastic³ curves. In a few cases cell counts or colloidal tests are lacking. Spinal puncture was performed as a routine after one or two courses of arsphenamin (from two to six months after admission),⁴ though in a few cases it was done much earlier or much later in the course of treatment. Puncture before treatment has been considered unjustifiable, partly because of the delay necessary in starting treatment, and partly because of the theoretical danger of transferring organisms from an infected blood stream to a non-infected cerebrospinal axis, either directly through the puncture wound with hemorrhage into the subarachnoid space, or by an alteration of the meningeal permeability.⁵

TABLE I.

The incidence of early neurosyphilis in primary and secondary syphilis.

	Number	Per cent.
Total cases	352	100
Acute syphilitic meningitis	2	0.56
Precocious vascular neurosyphilis	1	0.28
Neurorecurrences	19	5.3
Early asymptomatic neurosyphilis	72	20.4
Total early neurosyphilis	94	26.4

¹ Zimmermann, E. L.: Neurorecurrences, following treatment with Arsphenamin. Arch. Derm. and Syph., V, 723, (June) 1922. This paper deals with the neurorecurrences observed in this clinic, and contains a thorough review of the literature.

² It is recognized that nothing in this paper can be applied to the early detection of purely vascular neurosyphilis. The lesion in this type of case may be a small perivascular infiltration deeply buried in nervous tissue, and inaccessible to early clinical or laboratory methods of detection.

³ Keidel, A., and Moore, J. E.: Comparative Results of Colloidal Mastic and Colloidal Gold Tests. Arch. Neur. and Psych., VI, 163, (Aug.) 1921.

⁴ For five years, spinal puncture has been performed in the outpatient clinic, the patients being instructed to go home to bed for 48 hours. In that period, more than 2000 spinal punctures have been done, with no serious after-results. About half the patients suffer with puncture headache, but this unpleasant reaction is more than counter-balanced by the value of the information gained. Of course, this procedure is not advised if hospital beds are available.

⁵ Weed, L. H., Wegforth, P., Ayer, J. B., and Felton, L. D.: A Study of Experimental Meningitis. Monographs Rockefeller Inst. for Med. Research, No. 12, March 25, 1920.

Ninety-four of the 352 patients developed early neurosyphilis, and are subdivided as shown in Table I. Of these early neurosyphilitics, 76.6 per cent were asymptomatic. The importance of this sub-group is at once apparent.

In a preliminary communication⁶ a tentative division of early asymptomatic neurosyphilis into three groups was outlined, the grouping being based in part on the type of spinal fluid abnormalities (in 166 cases), and in part on the response of the various types to treatment. Further observation of this much larger series lends support to this tentative classification, and indicates that an appreciation of the grouping has a real prognostic value.

The first group of this classification includes those cases in which the spinal fluid abnormalities consist of

TABLE II.

Spinal Fluid findings of Group I, Early Asymptomatic Neurosyphilis.

Case	Cells	Glob.	Wassermann			Colloidal Gold	Colloidal Mastic
			0.2	0.4	1.0		
Zx2	8	±	0	0	0	1222110000	1000000000
Yx11	10	++	0	0	0	2211100000	2221000000
Jx20	8	±	0	0	0	1122222200	2100000000
Hx8	13	±	0	0	0	1100000000	2210000000
Bx2	9	+	0	0	0	1122211000	2210000000
Fx24	8	±	0	0	0	1111100000	2210000000
Fx6	21	+	0	0	0	0011100000	2210000000
Ex12	18	++	0	0	0	1222100000	2221100000
20	13	+	0	0	0	1221000000	
Gx30	7	++	0	0	0	1111100000	1110000000
Px21	6	±	0	0	0	0111100000	2100000000
Nx29	20	+	0	0	0	1122211000	1000000000
Hx28	8	±	0	0	0	0000000000	2222100000
6	8	±	0	0	0	0011000000	
T16	8	±	0	0	0	1221000000	
Lx16	15	+	0	0	0	0000000000	1111100000
M31	8	++	0	0	0	1110000000	
Kx18	10	+	0	0	0	1100000000	1122110000

* Keidel, A., and Moore, J. E.: Studies in Asymptomatic Neurosyphilis:—I. A Tentative Classification of Early Asymptomatic Neurosyphilis. Arch. Neur. and Psych., VI, 286, (September) 1921.

The standard of cell normality has been considered to be from 0 to 5 cells. From 6 to 10 is considered borderline, and such a fluid has not been classed as pathologic unless there was a concomitant increase in the globulin content. More than 10 cells per cu. mm. is definitely abnormal. This standard is based on the work of W. Schönfeld, Ueber Befunde in der Rückenmarksfliissigkeit bei nervengesunden Menschen. Deutsche Ztschr. f. Nervenhe., LXIV, 300, 1919.

* No stress is laid on alterations in pressure or on the isolated presence of a small amount of globulin, since both of these are frequently found in normal individuals. Both colloidal curves have been classed as negative if there was no change reading higher than 2.

* Justification for regarding these spinal fluid changes as evidence of neurologic damage is given by O. Fischer, (Die Anatomische Grundlage der Cerebrospinalen Pleozytose. Monatschr. f. Psych. u. Neur., XXVII, 512, 1910).

pleocytosis, usually slight,⁷ a slight increase in globulin content, but negative Wassermann reaction and colloidal tests (Table II).⁸ The discussion of treatment of this and other groups will be deferred till later in the paper, but it may be stated here that routine anti-syphilitic treatment suffices to clear up these abnormalities in practically all instances.⁹

In Group II are included those patients whose spinal fluids show a more marked pleocytosis, with cells between 10 and 100, but usually less than 50; the globulin content also is greater than in Group I; the Wassermann reaction may be either positive or negative, but if positive, fixation usually occurs only with large amounts of fluid; of the two colloidal tests, either or both may be

TABLE III.

Spinal Fluid findings in Group II, Early Asymptomatic Neurosyphilis.

Case	Cells	Glob.	Wassermann			Colloidal Gold	Mastic Colloidal
			0.2	0.4	1.0		
B4	56	+++	4	4	4	1123311000	
C13	98	+	1	3	4	1234421100	
M18	63	+++	4	4	4	1123332100	
Fx14	15	++	0	0	0	0011110000	3222000000
Ab14	10	+	0	0	0	222*32*11000	1100000000
Ru	12	+	0	0	0	0112110000	3211000000
Sy	28	+	0	0	4	1211100000	5543220000
Px22	29	±	0	0	4	1111000000	2210000000
Gx1	3	+++	0	0	4	1111110000	3221000000
Ax17	21	++	0	0	0	2445522100	2111000000
ix17	14	+++	4	4	4	2222200000	4322200000
Ix1	2	+	0	0	0	1233332000	1000000000
Bx29	21	+	0	0	1	1112333000	5432110000
C15	?	+	0	0	4	1234411000	
Mx20	6	±	0	0	0	1234411000	2210000000
121	16	++	0	0	4		
Kx9	14	+	0	4	4	1111100000	2210000000
Sx12	?	±	0	0	4	1222100000	2210000000
Di	18	++	0	0	0	2223222100	5543100000
Dm	16	±	0	0	0	2243100000	2211000000
Fx8	2	+	0	0	0	1211000000	3322100000
B19	19	±	4	4	4	1222100000	5543210000
Zx14	12	±	0	0	0	12*33100000	2100000000
Fx12	2	++	0	0	0	1111200000	3222200000
Kx2	24	+	0	0	4	0001100000	2210000000
Nx27	5	+	0	0	0	0134211000	2000000000
Cx23	16	+	0	0	0	0123100000	4322100000
Fx10	8	++	0	1	4	3433211000	5543200000
Bx15	?	+	1	4	4	1123110000	3222100000
Jx16	2	++	0	0	0	3333210000	3221000000
Px3	7	+	0	0	0	2332110000	3220000000
Px5	3	++	0	0	0	1133110000	3221000000
Dx15	40	+++	0	0	4	1123311000	
Dx5	?	+++	0	0	0	2233100000	2220000000
Nx20	4	++	0	0	0	0123320000	2210000000
Nx30	2	+++	0	0	4	1555555210	0033220000
Bb16	4	±	0	0	0	443*3321000	4321000000

positive, the gold being of the syphilitic zone type, and the mastic curve usually to 3, or rarely paretic. Table III shows the detailed findings in 37 cases. It is apparent that Group II is the most elastic of the three groups.

and that certain cases fall into this group, instead of into Groups I or III, only because of the results of the colloidal tests. It is for this reason that we do not consider a fluid examination complete, unless at least one and preferably both of the colloidal tests can be performed. In this group also a serologic and clinical "cure"¹⁰ may be obtained by slight modifications in the usual routine of anti-syphilitic treatment without the addition of intraspinal therapy.

Group III includes those cases in which the spinal fluid changes are of a more advanced type. The cell count is high, ranging from 50 to 200. The globulin content is markedly increased; the Wassermann reaction is positive with small amounts of fluid (0.2 cc. or less); and the colloidal gold and mastic curves are both paretic (Table IV). The abnormalities of this group are much more

TABLE IV.

Spinal Fluid findings in Group III, Early Asymptomatic Neurosyphilis.

Case	Cells	Glob.	Wassermann			Colloidal Gold	Colloidal Mastic
			0.2	0.4	1.0		
11	32	++++	4	4	4	5554310000	
13	91	+++++	4	4	4	5555540000	
77	58	+++++	4	4	4	5552110000	
90	68	++	4	4	4	5555431000	
O1	12	++++	4	4	4	5555321000	
X4	64	++	4	4	4	5555542000	
S32	18	+++	4	4	4	5554210000	
S47	28	++	4	4	4	5555553100	
Gx18	42	++++	4	4	4	5555533200	5543210000
Hx5	65	+++++	4	4	4	2211000000	5543210000
Jx1	98	++++	4	4	4	5555543000	5532100000
Qx27	150	+++++	4	4	4	5555442100	5555542100
Du	112	+++	4	4	4	5441100000	5532100000
Mo	156	+++++	4	4	4	5555520000	
Ze	131	+++++	4	4	4	5555554100	
Br	37	+++++	4	4	4	5555521000	
Ho	40	+++++	4	4	4	5555421000	

resistant to treatment than the preceding two; and practically no improvement results from the routine use of arsphenamin and the mercurials. Intraspinal therapy as an adjunct to routine treatment offers almost the only chance of a serologic cure.

The relative incidence of these three groups is shown in Table V. Of 67 patients with primary syphilis, 20.9 per cent had abnormal fluids (9.1 per cent fluid abnor-

malities in sero-negative, 26 per cent in sero-positive, primary syphilis). In the 263 patients with secondary syphilis, 22.1 per cent of the fluids were abnormal. If argument for the early diagnosis and vigorous treatment of primary syphilis were needed, it would be fur-

TABLE V.

Incidence of early asymptomatic neurosyphilis in primary and secondary syphilis.

	Total	Normal	Abnormal	Fluids falling into Groups		
				I	II	III
Primary	67	53	14 (20.9%)	3	9	2
Secondary	263	205	58 (22.1%)	15	28	15
Total	330	258	72 (21.8%)	18	37	17

nished by these figures; although it is evident that in some instances neurologic invasion may occur no matter how early treatment is begun.

Under the conditions of this study (examination of the spinal fluid after a considerable amount of treatment) it appears that in Group II we have the most frequent type of abnormal fluid. Of 72 cases of asymptomatic neurosyphilis, 25 per cent fall into Group I; 51.3 per cent into Group II; and 23.6 per cent into Group III. From the reports of other workers¹¹ on patients with untreated syphilis, it would seem that the minor abnormalities of Group I are much more frequent. Treatment, as we have employed it, may be considered to have accomplished either one or both of two things:—a rapid disappearance of the Group I changes, so that when examined the fluid appears normal; or the opposite effect of intensifying the minor abnormalities, so that a patient who might belong to Group I before treatment falls into Groups II or III after treatment. This assumption, for which some proof exists, constitutes an additional reason to the two already given for delaying puncture until after the administration of anti-syphilitic treatment.

The most important problems in the present day study of neurosyphilis concern the time and manner of invasion of the central nervous system. The scientific aspect of the latter is overshadowed by the practical importance of the former. If it were possible to be reasonably sure of the time limits within which neurologic invasion might occur, the prevention of clinical neurosyphilis by means of its early detection would rest on firmer ground. The best available method of approach to this problem is by a study of the incidence of spinal fluid abnormalities at varying intervals after infection. If invasion of the nervous system occurs within the first months of the infection, one would expect a gradually increasing incidence, reaching a peak during the first or second years, and thereafter remaining at approximately the same

¹⁰ The word "cure" is here and elsewhere in this paper used to mean that the patient remains clinically well, and that the serology of the blood and spinal fluid becomes negative and remains so for the period of observation. It is not used in the sense of eradication of the last remaining organism. Hereafter, the quotation marks will be dispensed with.

¹¹ Particularly G. L. Dreyfus, (Münch. Med. Woch., LXVII, 1369, 1920) who finds from 70 to 80 per cent of abnormal fluids in untreated secondary syphilis. See also U. J. Wile, and C. K. Hasley: Involvement of Nervous System during Primary Stage of Syphilis, Jour. Am. Med. Assn., 76, 8, (Jan. 1) 1921.

TABLE VI.

The Incidence of Abnormal Spinal Fluids in Primary and Secondary Syphilis, showing the duration of infection in months at the time of puncture:—a comparative study.

Duration of syphilis in months	Fordyce and Rosen			This study			Composite results		
	Normal fluids	Abnormal fluids	Per cent abnormal	Normal fluids	Abnormal fluids	Per cent abnormal	Normal fluids	Abnormal fluids	Per cent abnormal
0—3	20	2	9.0	15	3	16.6	35	5	12.5
4—6	43	9	17.3	37	7	15.9	90	16	15.1
7—9	30	10	25.0	25	10	28.5	55	20	26.6
10—12	29	15	34.1	36	11	23.4	65	26	28.5
13—15	19	13	40.6	42	12	22.2	36	13	26.5
16—18							25	12	32.4
19—21	38	15	28.5	34	5	12.8	24	8	25.0
22—24							48	12	20.0

level. If, on the contrary, this invasion may take place at any time, the curve of incidence should be a constantly increasing one.

In Table VI and Chart I, we have compared the results obtained by Fordyce and Rosen¹² in 243 cases with 237 comparable cases from the present study, and each of these results with a combination of the two series. These patients were all punctured within the first two years of the disease, and the cases are divided into three month periods, showing the duration of infection at the time of puncture. The conditions as to treatment are similar in the two series of cases:—in each instance puncture was deferred until after some treatment, usually one or two courses. The graphic presentation in the chart shows a rapid rise in the incidence of abnormal spinal fluids, which, according to Fordyce and Rosen, reaches its peak

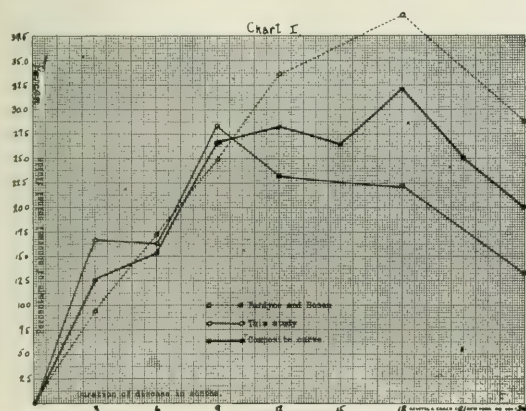
18 months after infection; according to the present series this peak is reached by the ninth month. A consolidation of the two series provides an intermediate curve, probably more accurate than either alone because of the larger number of cases, in which the first peak is reached at the end of 12 months. Thereafter, except for a secondary rise at the eighteenth month, the incidence of abnormalities remains at about the same level. This is further shown in Table VII, in which our own cases are arranged according to the duration of the disease in years at the time of spinal puncture. If the probable statistical error is taken into account, there is little or no variation

TABLE VII.

Incidence of spinal fluid abnormalities, arranged according to the duration of infection in years.

Year	Total fluids	Normal	Abnormal	Per cent abnormal	Fluids falling into Groups		
					I	II	III
1	149	99	35	23.3	11	17	7
2	95	69	19	20.0	4	9	6
3	27	20	7	25.9	1	5	1
4	28	20	8	28.5	2	2	4
5	19	16	3	15.7	1	—	2
6	9	8	1	11.1	—	1	—

¹² Fordyce, J. A. and Rosen, I.: Laboratory Findings in Early and Late Syphilis:—a Review of One Thousand and Sixty-Four Cases. Jour. Am. Med. Assn., 77, 1696, (Nov. 26) 1921.



in the different years.¹⁴ These results are somewhat complicated by the amount of treatment administered, as in general those patients whose disease at the time of puncture was more than two years old had received more treatment than the remainder. This factor may account for the slightly lower percentages in the later duration groups.

Recent experimental work on syphilis has provided information regarding the course of the infection on which an interpretation of these results may be based. Within a few days after infection *Treponema pallidum* may be recovered from the lymph glands¹⁴ and from the heart's blood of experimental animals.¹⁵ In human syphilis the blood is infectious during the primary and secondary stages, though organisms are probably present only in small numbers. When the period of latency has been reached, it is non-infectious except in rare instances.¹⁶ From the lymph glands, on the contrary, virulent organisms may be recovered at any time during the course of the disease. This has been interpreted to mean that the lymph nodes function as reservoirs for the organisms.

It is probable that at the time of infection, dissemination of organisms takes place from the lymphatic system to the peripheral blood stream. The blood is not an ideal medium for their growth or continued residence, and as soon as possible, they leave it for more favorable surroundings. After a second period of incubation in their new locations, the tissues react with the secondary outbreak. At this time, it is probable that an immune reaction takes place,^{17, 18} so that with the spontaneous regression of secondary lesions, the vast majority of the organisms are destroyed by the natural defenses of the body. This reaction may permit the destruction of any further organisms which may be discharged from the lymphatic system to the blood; or the actual discharge may perhaps be prevented by the local tissue reaction in the lymph glands, walling off the organisms and obstructing their egress. When this has been accomplished, the appearance of lesions of the secondary type ceases and latency ensues. The length of time necessary to bring about this result probably varies from three to twelve months.

If, during the first months of the disease, organisms are more or less constantly present in the blood, it is difficult to understand how invasion of the nervous system fails to occur. Evidence is gradually accumulating to show that such an invasion does take place in the majority of all cases,¹⁹ probably by vascular involvement of the meninges, parenchyma, or both. Whether or not clinical neurosyphilis will develop must partially depend on the extent to which the individual patient reacts against his infection.²⁰ That most patients do succeed in dealing spontaneously with this neurologic invasion is evidenced by the comparatively small number of clinical neurosyphilis.

The presence of spinal fluid abnormalities may depend on the length of time the organisms have been resident in nervous tissue. If puncture is performed at about the time that invasion occurs, the fluid may be normal, whereas if it had been delayed a few weeks or months, during which the nervous tissues had time to react, definite abnormalities might be present. For example, it is shown by the study of untreated sero-negative primary,

¹⁴ Moore, J. E.: The Cerebrospinal fluid in Treated Syphilis. Jour. Am. Med. Assn., **76**, 769, (March 19) 1921. A study of 451 cases of late (tertiary and latent) syphilis without neurologic abnormalities showed no higher incidence of abnormal spinal fluids than in early syphilis. The conclusion was drawn that "after the disease is presumably well entrenched, about the same relative number of patients show spinal fluid abnormalities, no matter how long the disease has existed, or by what lesions it manifests itself."

¹⁵ Pearce, L. and Brown, W. H.: A Study of the Relation of *Treponema pallidum* to Lymphoid Tissues in Experimental Syphilis. J. Exp. Med., XXXV, 39, (Jan.) 1922.

¹⁶ Brown, W. H. and Pearce, L.: A Note on the Dissemination of *Spirochaeta pallida* from the Primary Focus of Infection, Arch. Derm. and Syph., II, 470, (Oct.) 1920. Also Ebersson, F.: Dissemination of *Spirochaeta pallida* in Experimental Syphilis, Arch. Derm. and Syph., III, 111, (Feb.) 1921.

¹⁷ Engman, M. F. and Ebersson, F.: A Biologic Study of Latency in Syphilis. Arch. Derm. and Syph., III, 347, (April) 1921. This article contains a review of the literature on the infectivity of body fluids.

¹⁸ The question of immunity in syphilis is still *sub judice*, but enough clinical and laboratory evidence exists to make certain the presence of a defensive reaction to infection. The nature of this reaction is as yet unknown. For a discussion of this point, see the article by Engman and Ebersson, (footnote 16).

¹⁹ Brown W. H. and Pearce, L.: The Resistance (or Immunity) Developed by the Reaction to Syphilitic Infection. Arch. Derm. and Syph., II, 675, (Dec.) 1920. Experimental Production of Clinical Types of Syphilis in the Rabbit. Arch. Derm. and Syph., III, 254, (March) 1921.

²⁰ This statement is based on the high percentage of abnormal spinal fluids found by various workers in untreated early syphilis, and on the demonstration of treponemes in the spinal fluid of early syphilis with otherwise normal fluids. This has been accomplished by Marinesco and Minea (Acad. de Sc., Sem. Méd., 1914, p. 357), Steiner (Neurol. Centralb., 1914, XXXIII, 132), and others. Brown and Pearce have demonstrated organisms in the spinal fluid of recently infected rabbits.

A. Steiner, (Moderne Syphilisforschung und Neuropathologie. Arch. f. Psych., 1913, LII, 1), found pathologic alterations attributable to syphilis in the nervous systems of 16 of 31 rabbits. Six of 8 animals with generalized syphilis showed central nervous system involvement.

²¹ It has been suggested, on the theory of immune reactions which may vary in different body tissues, "that all tissues are not equally protected by the general reaction which occurs during the early stages of a syphilitic infection, and that certain tissues which fail to receive this protection, although less susceptible to injury or infection than other tissues, may be capable of only a slight degree of self-protection. This is undoubtedly the case in the experimental animal, and if similar conditions obtain in man, such conditions as neurosyphilis might be explainable on this basis." Brown, W. H. and Pearce, L.: Arch. Derm. and Syph., March, 1921, III, 254.

sero-positive primary, and secondary syphilis, that the incidence of fluid abnormalities rises with a ratio for these examples of about one to three to four, in the order named.²¹ This factor must also be considered in connection with the grouping outlined in this paper. A patient classed in Group I, if punctured three months after infection, might fall into Groups II or III if the fluid were studied six months later. The optimum time for the detection of the largest number of cases with abnormal fluids, provided no treatment were administered, would doubtless be from the twelfth to the eighteenth month after infection (see Chart I). The influence of anti-syphilitic treatment, however, makes this delay unnecessary. If treatment is regular and adequate, the abnormalities already present (except for Group III) will rapidly disappear, and opportunity for further neural invasion will be prevented. On the other hand, if a small amount of treatment is followed by a lapse, there is, as will be shown, an increased liability to neurosyphilis. Puncture should be employed as a routine measure, therefore, in all patients with early syphilis at the end of the first or second course of regular treatment. If the fluid is completely negative at this time, puncture need not be repeated until the completion of treatment and observation. Should a lapse in treatment occur, however, the case should be managed as if it were a fresh infection, and puncture be repeated again following the resumption of treatment.²²

TABLE VIII.

Arrangement of cases by race and sex.

		Total fluids	Normal	Abnormal	Fluids falling into Groups		
					I	II	III
Colored		72	61	11 (15.1%)	4	5	2
	Male						
	Female	51	43	8 (15.6%)	4	4	—
	Total	123	104	19 (15.4%)	8 (6.5%)	9 (7.3%)	2 (1.8%)
White		145	109	36 (24.8%)	5	21	10
	Male						
	Female	62	45	17 (27.4%)	5	7	5
	Total	207	154	53 (25.6%)	10 (4.8%)	28 (13.5%)	15 (7.2%)
Males both races		217	170	47 (21.6%)	9 (4.1%)	26 (11.9%)	12 (5.5%)
Females both races		113	88	25 (22.1%)	9 (7.9%)	11 (9.7%)	5 (4.4%)

It is now necessary to consider other factors which may influence invasion of the central nervous system. In Table VIII the available data are rearranged by race and sex. There is evident a wide difference between the colored and the white races in their susceptibility to early neurosyphilis, the incidence being 15.4 per cent as compared with 25.6 per cent. Cases of the Group I type are about equally frequent in the two races, but Group II is almost twice as common in whites and Group III four times as common. In other words, the negro is not only less prone to neural invasion than the white, but when his central nervous system is invaded, his defensive reactions enable him to deal with the infection so that, judging from the spinal fluid changes, his neurosyphilis is consistently milder than is that of the white. This observation is in accord with statistical data regarding the comparative incidence of paresis and tabes, which are rare in the colored race.²³

No difference is apparent between the sexes, of either or both races. Early neurosyphilis is as common in women as in men, and the incidence of the various groups is approximately the same. This is a surprising result, in view of the fact that in 763 unselected cases of clinical neurosyphilis, there were four times as many men as women (paresis five times, tabes six times, and cerebrospinal syphilis about three times as frequent). The

TABLE IX.

Showing the incidence of spinal fluid abnormalities according to the age at the time of infection.

Age group	Total cases	Negative	Abnormal	Per cent Abnormal
0—15	4	4	—	—
16—20	67	55	12	17.8
21—25	105	80	25	17.9
26—30	77	60	17	22.0
31—35	48	35	13	27.0
36—40	10	9	1	10.0
41—65	19	15	4	21.0
0—30	253	199	54	21.3
31—65	77	59	18	23.3

²¹ With, C.: Studies on the Different Reactions in the Cerebrospinal fluid in Cases of Syphilis. *Brain*, 1918, XL, 403. Wile, U. J. and Marshall, C. H.: A Study of the Spinal Fluid in One Thousand Eight Hundred and Sixty-Nine Cases of Syphilis in all Stages. *Arch. Derm. and Syph.*, March, 1921, III, 272.

²² Moore, J. E.: The Genesis of Neurosyphilis. *Arch. Derm. and Syph.*, July, 1921, IV, 55.

²³ Zimmermann, E. L.: A Comparative Study of Syphilis in Whites and in Negroes. *Arch. Derm. and Syph.*, July, 1921, IV, 75.

explanation is not apparent, though the influence of pregnancy on the disease²⁴ may play some rôle.

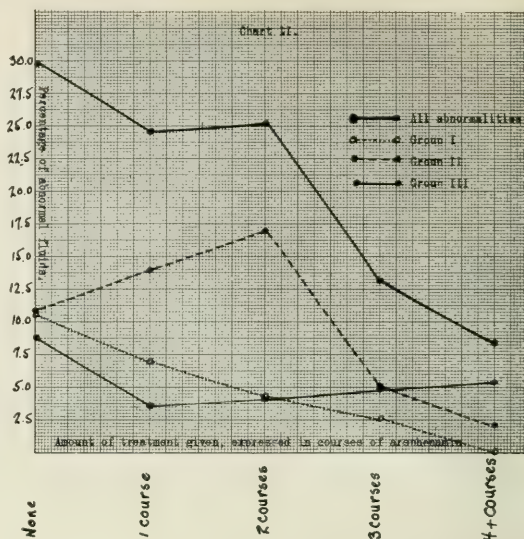
Table IX shows the incidence of spinal fluid abnormalities of all groups arranged by age at the time of infection. There seems to be no special liability for early neurosyphilis to occur in any particular age group, thus disposing of the often expressed theory that if syphilis is acquired in old age the central nervous system is more liable to invasion than early in life. The variations in age groups in this series are well within the limits of statistical error.

In our preliminary paper⁶ it was stated that the incidence of early neurosyphilis was the strongest argument in favor of the adequate treatment of all syphilis, and we presented a series of 151 cases in which the inter-relation of spinal fluid abnormalities and the amount of treatment given prior to the examination of the fluid was studied. The further evidence provided by the present much larger series corroborates our early opinion. These data are shown in Table X and Chart II. Unless treatment is continuous over a comparatively long period of

TABLE X.
Spinal fluid abnormalities in primary and secondary syphilis as influenced by treatment.

Amount of treatment	Total number of fluids examined	Negative	Positive	Per cent positive	Fluids falling into Groups		
					I	II	III
None or very little	57	40	17	29.8	6 (10.5%)	6 (10.5%)	5 (8.7%)
Six doses arsphenamin ± mercury	115	87	28	24.3	8 (6.9%)	16 (13.9%)	4 (3.4%)
Twelve doses arsphenamin plus mercury	70	52	18	25.7	3 (4.2%)	12 (17.1%)	3 (4.2%)
Eighteen doses arsphenamin plus mercury	38	33	5	13.1	1 (2.6%)	2 (5.2%)	2 (5.2%)
Twenty-four or more doses arsphenamin plus mercury	50	46	4	8.0	—	1 (2.0%)	3 (6.0%)

time, no marked effect is apparent. One or two courses of arsphenamin, with or without mercury, accomplish little. When three courses are given, and particularly if treatment is regular, a decided drop in the incidence of abnormalities occurs; and after four or more courses the



abnormal percentage is very low. Reference to Chart II shows that the number of cases of the Group I type is rapidly decreased by treatment, the fall in percentage being from 10.5 when no treatment or very little is given, to zero when four or more courses are administered. A curious change is seen in the curve for the Group II type. Instead of an early and continuous drop in the incidence of this group, there is a sustained rise from 10.5 per cent, after no treatment or very little, to 13.9 per cent after one course, and to 17.1 per cent after two courses of treatment. At this point the incidence begins to decrease; after three courses it is 5.2 per cent, and after four, 2 per cent. The interpretation of this phenomenon is difficult; but it is probably closely related to the question of the influence of a lapse in treatment in causing the appearance of precocious neurosyphilis. Two probable deductions may be made from this curve:—(1) a number of patients, who, if punctured before treatment, would have shown normal or Group I fluids, later fall into Group II because of the influence of insufficient treatment followed by a lapse. In other words, they represent asymptomatic neurorecurrences, and fail to develop symptoms only because the lesion is in a "silent area," not involving cranial nerves or other important structures;²⁵ (2) these cases plus the ones originally in

²⁴The mild course of syphilis in pregnant women is a well established clinical fact. Experimental evidence of the ability of pregnancy to suppress syphilitic manifestations has been furnished by Brown and Pearce:—On the Reaction of Pregnant and Lactating Females to Inoculation with *Treponema pallidum*—a Preliminary Note. *Am. J. Syph.*, Oct., 1926, IV.

²⁵Gennerich, W.: *Die Syphilis des Zentralnervensystems* Berlin, Julius Springer, 1921.

Group II are rapidly cleared up serologically after further adequate treatment.

Cases of the Group III type are apparently uninfluenced by treatment, no matter how far prolonged. Their incidence is almost as high following four or more courses of arsphenamin plus mercury as when no treatment at all is given. It is worthy of note, that, as the period of treatment is prolonged, the curve representing that of all abnormalities begins to approach that of the Group III type; so that presumably, if treatment were continued long enough, Groups I and II would disappear, and the Group III curve would replace that for all abnormalities, continuing in approximately a straight line. When the treatment of these three abnormal groups is discussed in detail, evidence supporting these interpretations will be provided.

It is of interest to examine the influence of a lapse in treatment on the incidence of these various abnormal groups.²⁶ The data on this point are shown in Table XI. Under the heading of "regular" treatment are classed all patients in whom spinal puncture was performed either before any treatment whatever was given, or immediately following the administration of treatment which was carried out in a regular manner. Under the heading "lapsed" are included all patients who, after taking from one to twelve doses of arsphenamin either with or without mercury, voluntarily discontinued treatment and were punctured some weeks, months, or years later. The incidence in the latter class of patients is three times as high as in the former. Still more significant is an analysis of the types of abnormalities found. From this angle the table shows that under the condi-

TABLE XI.

The Effect of a Lapse early in treatment in causing the development of spinal fluid abnormalities.

Treatment	Total cases	Negative	Positive	Per cent positive	Fluids falling into Groups		
					I	II	III
Regular	206	181	25	12.1	6 (2.9%)	17 (8.2%)	2 (0.9%)
Lapsed	124	77	47	37.9	12 (9.6%)	20 (16.1%)	15 (12.0%)

tions of lapsing treatment, as compared with regular treatment, the percentage of Group I cases is three times as high, of Group II twice as high, and of Group III twelve times as high. It is demonstrated (Table X and Chart II) that regular treatment decreases the incidence of two of these group types. Irregular treatment, on the contrary, not only increases their incidence very markedly, even to a higher percentage than that observed when no treatment at all has been given, but what is much more portentous of gravity in the situation, this faulty management often intensifies the character of the

spinal fluid changes. The explanation of this phenomenon is identical with that advanced to account for the mechanism of neurorecurrences.²⁷

The existence of a neurotropic strain of organism as a possible etiologic factor was also investigated.²⁸ In this series of 72 cases of asymptomatic neurosyphilis it was possible to examine the marital partner by the standards laid down in our first paper (history, physical and neurologic examination, blood Wassermann and a study of the

TABLE XII.

The Incidence of Conjugal Neurosyphilis in the Partners of Asymptomatic Neurosyphilitics.

Case no. of patient with asymptomatic neurosyphilis	Group	Sex of partner	Partner syphilitic	Partner neuro-syphilitic	Type of neurosyphilis in partner
20	I	M	Yes	No	
C 13	II	M	Yes	No	
M 18	II	M	Yes	Yes	Cerebrospinal syphilis; duration of infection 3 years.
Px 22	II	F	No	No	
Gx 1	II	F	Yes	No	
Fx 8	II	F	Yes	Yes	Asymptomatic neurosyphilis; duration of infection 10 months.
Jx 16	II	F	No	No	
D 15	II	F	Yes	No	
11	III	M	Yes	No	
Hx 5	III	M	Yes	Yes	General Paresis; duration of infection 12 years.

²⁶ Fraser, A. R. and Duncan, A. B. G.: A Possible Explanation of the Increased Incidence and Early Onset of Neurosyphilis. *Brit. J. Derm. and Syph.*, 1921, XXXIII, July, 251; Aug.-Sept., 281.

²⁷ This reaction is not, as has been assumed by Klauder (Early Neurosyphilis Asymptomatica, *Am. J. Syph.*, 1919, III, 559), a Herxheimer reaction, as we understand the limitations of this term. The Jarisch-Herxheimer reaction follows only the first injection of a series of arsphenamin, occurs within a few hours after the injection, and consists of an acute tissue reaction in a syphilitic lesion due to the rapid destruction of the spirochetes which it contains. The type of response referred to above, on the contrary, occurs in the central nervous system or elsewhere some weeks after the cessation of treatment, the interval being occupied by the multiplication of organisms in the focus and the reaction of the tissues to their presence. The length of time necessary to produce this result corresponds closely with the incubation period of a lesion at the point of first inoculation.

²⁸ Moore, J. E. and Keidel, A.: Studies in Familial Neurosyphilis:—I. Conjugal Neurosyphilis. *Jour. Am. Med. Assn.*, July, 1921, 77, 1.

cerebrospinal fluid) in ten cases, with positive results in only three. A summary of these data appears in Table XII. Eight of the marital partners of the patients in this series had syphilis; but only three were neurosyphilitic; These were partners of two Group II patients and one Group III patient, respectively. While it is of course recognized that this series is too small to permit any definite conclusions, there is no suggestion that strain of organism is here an important factor.

That many patients with clinical neurosyphilis give a history of very slight or no general manifestations of secondary or tertiary syphilis is a fact beyond dispute.²⁹ This has led to the conclusion that the greater the early tissue reaction, the smaller the liability to late grave manifestations. In other words, those patients who react to a recent infection with a violent secondary outbreak are assumed thereby to develop a defense, probably of the type of tissue immunity, against later lesions. So far as neurosyphilis is concerned, its lower incidence in the colored as compared to the white race lends some support to this view. It has been frequently shown that secondary syphilis in negroes is more extensive and more severe than in whites. In order to discover if this factor played any rôle in the production of early asymptomatic neurosyphilis, the early lesions of the 72 patients in this series have been studied. It is obviously impossible to decide upon any strict basis of comparison for the extent or severity of early secondary syphilis because of the individual equation of the examiner. We have therefore subdivided the types of early lesions seen into three groups:—mild, average, and severe. Primary syphilis has been considered as mild if there was a single diminutive chancre, or if the proper period for the appearance of secondary

lesions was much overdue, and as severe, if there was an extensive single or multiple lesion. Mild secondary syphilis is that manifested by a sparse rash of a macular or papular type, or by a few mucous lesions only; those cases classified as severe were all specifically noted to have very profuse rashes. All other cases are classed as average. On this basis, Table XIII shows that in the 72 early neurosyphilitic patients, mild early manifestations were three times as frequent as in 174 unselected cases of early syphilis, and that this increase takes place at the expense of the average group. It is noteworthy that 13.8 per cent of these neurosyphilitic patients, however, had extensive secondary outbreaks. Although, therefore, failure to react vigorously to a recent infection seems to predispose to neurosyphilis, a violent secondary outbreak does not necessarily protect the patient against it. There is nothing in this fact to strengthen the argument of the advocates of a neurotropic strain of organism.³⁰

As brought out by this and similar studies³¹ there are certain clinical aids to the recognition of this type of early neurosyphilis. These are various subjective and objective symptoms, and the behavior of the blood Wassermann reaction. The subjective symptoms consist of headache, which may be quite mild, but is often severe enough to cause the patient sleepless nights; insomnia without headache; a complaint of vague neuralgic pains over the whole body, which resemble the usual prodromata of various acute infections, especially influenza; giddiness, never severe enough to cause staggering gait or falling, and always, in those cases which we have had examined, without objective lesions in the vestibular branch of the eighth nerve; and nervousness. In Table XIV it is shown that about 30 per cent of those patients with abnormal fluids complained of one or more of these symptoms; while similar complaints were found in only 9.7 per cent of those with negative fluids. It is notable

TABLE XIII.

Character of Early Cutaneous Lesions of Syphilis in Early Asymptomatic Neurosyphilis.

Group	Total cases	Character of early cutaneous lesions		
		Mild	Average	Severe
I	18	5	11	2
II	37	15	16	6
III	17	7	8	2
Total	72	27 (37.5%)	35 (48.6%)	10 (13.8%)
Unselected cases of early syphilis	174	23 (13.2%)	123 (70.6%)	28 (16.0%)

²⁹ Fournier, A.: (*Paralyse Générale et Syphilis*, Paris, Masson et Cie., 1905) was able to follow personally the evolution of the disease from chancre to general paresis in 83 cases. Of these, 70 had only a fleeting roseola or mucous patches or alopecia; 8 had moderate secondary lesions; and 3 had tertiary lesions. Two patients showed no specific manifestations except the chancre. On the other hand, of 243 cases of severe secondary syphilis followed for many years, not one showed symptoms of general paresis or tabes.

³⁰ Indeed, Fournier found (*Traité de la Syphilis*, Paris, J. Rueff, 1906, vol. II, part I, p. 7) the same phenomenon for tertiary lesions of any type. The figures are as follows: of 2188 cases of all types of tertiarism, 90.9 per cent were preceded by mild, very mild, or no secondary symptoms; 7.2 per cent by a moderate, and 1.8 per cent by a severe secondary outbreak.

³¹ Moore, J. E.: *The Cerebrospinal Fluid in Treated Syphilis*. Jour. Am. Med. Assn., 76, 769 (March 19) 1921. Fordyce, J. A.: Importance of Recognizing and Treating Neurosyphilis in the Early Period of the Infection. Am. J. Med. Sc., CLXI, 313, (March) 1921. Schou, H. J.: Nervelesies ved tidlig Syfilis og deres Behandling. Hosp.—Tid., LXIII, 57, 1920; abstr. in Med. Abstr. and Rev., IV, 61, (April) 1921.

TABLE XIV.

The Incidence of Neurologic Symptoms and Minor Neurologic Signs in Early Syphilis.

C. S. F.	Subjective signs			Objective signs		
	Absent	Present	Per cent in which present	Absent	Present	Per cent in which present
Negative	233	25	9.7	235	23	8.9
Abnormal	50	22	30.5	54	18	25.0
Group I	14	4	22.2	12	6	33.3
Group II	28	9	24.3	30	7	17.9
Group III	8	9	52.9	12	5	29.3

that more than half of the Group III cases had one or more of these complaints, usually headache.

The objective signs are, in our experience, almost wholly confined to minor pupillary and reflex changes. Careful sensory examinations, comparable to those carried out by some English observers,³² have not been made. The pupillary changes are all mild in degree, and consist of slight myosis or mydriasis, inequalities, irregularities, or some incompleteness or sluggishness of the direct or consensual light reaction.³³ Occasionally these phenomena have been noted to develop in this class of patients while under observation. The reflex changes are usually confined to the deep reflexes, most commonly the knee and ankle jerks, and consist only of some exaggeration or sluggishness, or more rarely of inequality. Twenty-five per cent of patients with abnormal fluids showed one or more of these neurologic changes; while 8.9 per cent of those with negative fluids showed similar manifestations.

It is obvious that both these subjective and objective signs may, and frequently do, occur in normal non-syphilitic individuals, as well as in syphilitics in whom no other neurologic damage can be demonstrated. Their presence, therefore, is no more than presumptive evidence of the existence of neurosyphilis. But any or all of them, occurring in a syphilitic patient, should direct the suspicions of the examiner toward this possibility.

³²Head, H. and Fearnside, E. G.: The Clinical Aspects of Syphilis of the Central Nervous System in the Light of the Wassermann Reaction and Treatment with Neosalvarsan. *Brain*, XXXVII, 1, (Sept.) 1914. This is a splendid paper which, in this country, has not received the attention it deserves.

³³Nonne, M.: Über die Bedeutung der Liquoruntersuchung für die Prognose von isolierten syphilitischen Pupillenstörungen. *Deut. Ztschr. f. Nervenb.*, LI, 155, 1914.

³⁴Stokes, J. H. and Busman, G. J.: A Clinical Study of Wassermannfast Syphilis. *Am. J. Med. Sc.*, CLX, 658, (Nov.) 1920.

We have frequently found, as have others,³⁴ that a persistently positive blood Wassermann reaction could be explained on the basis of neurosyphilis, and have therefore arranged this material to discover if possible the inter-relation of the two factors. On the basis of about 1000 cases of early syphilis, we anticipate an early reversal of the blood Wassermann reaction. As a matter of fact, a permanently negative blood Wassermann occurs by the sixth weekly dose of arsphenamin in the majority of all such cases. If the blood Wassermann is still positive after a second course of arsphenamin plus interim mercury, we begin a careful search for the cause, paying particular attention to the osseous, cardio-vascular, and central nervous systems. Reference to Table XV shows that after one course of arsphenamin without mercury,

TABLE XV.

Spinal Fluid Abnormalities in Primary and Secondary Syphilis as compared with Blood Wassermann Reaction and Treatment.

	Blood Wassermann Reaction	Total Number fluids ⁴	C. S. F. Normal	C. S. F. Abnormal	Per cent abnormal fluids	Fluids falling into Groups		
						I	II	III
After one course of treatment	Positive	32	21	11	34.3	5	4	2
	Negative	101	77	24	23.7	7	15	2
	Total	133	98	35	26.3	—	—	—
After two or more courses of treatment	Positive	17	11	6	35.2	1	2	3
	Negative	141	119	22	15.6	3	12	7
	Total	158	130	28	17.7	—	—	—

the blood Wassermann remained positive in 21.4 per cent of all cases with negative spinal fluids, while in the group of those with abnormal fluids it was positive in 31.4 per cent. (Naturally, all patients punctured before treatment are excluded from this table.) Expressed somewhat differently, of those patients with a persistently positive blood Wassermann reaction, 34.3 per cent had abnormal fluids, as compared with 23.7 per cent when the blood test had become negative.

After two or more courses of arsphenamin plus mercury, the figures are more impressive. Of 17 cases with a persistently positive blood reaction, 35.2 per cent showed

abnormal spinal fluids, while only 15.6 per cent of the negative blood cases were abnormal in this respect. In other words, 21.4 per cent of the early neurosyphilitics had a blood Wassermann difficult to reduce, whereas this phenomenon was present in only 8.4 per cent of the cases with a negative spinal fluid. These statistics demonstrate that to the list of subjective and objective signs already detailed must be added the factor of a stubborn blood Wassermann reaction; and that this, when present, should at once arouse the suspicion of neurologic invasion.

The Treatment of Early Asymptomatic Neurosyphilis.—When the classification of these cases was discussed, it was stated that the grouping was determined as much by the results of treatment of the various groups as by the type of spinal fluid abnormalities. The Group I type, with only minor abnormalities in the fluid, usually yields readily to routine anti-syphilitic treatment, provided this is carried out for a sufficiently long period of time. Unfortunately, too few cases of the present series have been consecutively treated to furnish convincing statistical proof of this point. This statement is, therefore, based on our experience with patients who miss falling into the classification under discussion by virtue of the longer duration of their disease, on the rapidly falling curve of incidence of Group I cases after adequate treatment (see Chart II), and on the evidence of other workers. Not only do these minor abnormalities disappear, but the patients remain clinically and serologically well.

Thirteen patients in our Group II have been under treatment for a sufficient length of time to allow discus-

sion of the results. The essential data are summed up in tabular form (Table XVI) in order to economize space. Of these cases, one (Px 22) has disappeared from observation; the remaining twelve are well after periods of observation ranging from 8 to 36 months after the first discovery of spinal fluid abnormalities. Eight patients have always been, and have remained, symptom-free. In all five instances in which various subjective symptoms were complained of, these rapidly disappeared. Six patients were found to have the minor neurologic changes described, and in five these objective signs have remained unaltered; while in one case (Ix 17), marked exaggeration of all deep reflexes has disappeared, leaving normal reflexes at present. If, as is probable, these slight abnormalities are evidence of beginning anatomical damage, it is not surprising that treatment usually failed to effect their disappearance.

From the standpoint of the blood Wassermann, all patients have been rendered sero-negative and have remained so. The cyto-biology of the cerebrospinal fluid is shown in the table. In most instances there were several examinations of the fluid made, only the last of which is shown. A negative result was often obtained much earlier than appears in the chart. In ten of the thirteen cases, a serologic cure¹⁰ has been obtained. In one instance (B 19) the cyto-biology of the fluid was markedly improved after 15 months' treatment, while in two cases (Px 22 and Kx 9) it was somewhat worse after smaller amounts of treatment. In each of these two cases the treatment given was very scant and was especially deficient in mercury.

The treatment given to all patients in this group, with

TABLE XVI.
The Results of Treatment of Early Asymptomatic Neurosyphilis—Group II.

Case No.	Duration of disease on admission	Treatment before first spinal puncture.	Lapse in treatment	Interval admission to spinal puncture	First C. S. F. results					Treatment after first puncture		Last C. S. F. results					Time elapsed since first puncture	Clinical result	Serologic result	Remarks	
					Cells	Glob.	Wa. R.	Gold	Mastic	Arsphenamin	Mercury	Cells	Glob.	Wa. R.	Gold	Mastic					
																					0.2
B 4	8 Mos.	5x0.6 Neo+Hg.	6 Mos.	7 Mos.	56	+++	4 4 4	1123311000	—	30x0.4	16 Weeks	1	+	0	0	0	1111100000	2210000000	19 Mos. Well	Cure	
C 13	2 Mos.	6x0.3	0	1.5 Mos.	98	+	1 3 4	1234421100	—	10x0.3	16 Weeks	8	0	0	0	0	1121100000	—	22 Mos. Well	Cure	
M 18	11 Mos.	12x0.3 & 20 wks. Hg.	0	7 Mos.	63	+++	4 4 4	—	—	32x0.3	16 Weeks	7	±	0	0	0	1111000000	2111000000	22 Mos. Well	Cure	
Fx14	2 Wks.	6x0.3	37 Mos.	38 Mos.	15	++	0 0 0	0011110000	3222000000	18x0.4	18 Weeks	2	+	0	0	0	1112111000	2100000000	12 Mos. Well	Cure	
Px22	5 Mos.	6x0.4+30 l. M. Hg.	3 Mos.	5 Mos.	29	±	0 0 4	1111000000	2210000000	12x0.4	4 Weeks	29	+	2	4	4	1122200000	1000000000	4 Mos. —	Worse	
Gx1	4 Mos.	12x0.4+6 wks. Hg.	0	4 Mos.	3	+++	0 0 4	1111110000	3221000000	22x0.4	8 Weeks	1	+	0	0	0	2211100000	2220000000	11 Mos. Well	Cure	
Ax17	7 Wks.	24x0.4+22 wks. Hg.	0	11 Mos.	21	++	0 0 0	2435522100	2111000000	14x0.6 (Neo)	16 Weeks	4	+++	0	0	0	1121000000	2222100000	14 Mos. Well	Cure	
Ix17	7 Mos.	None	—	0	14	+++	4 4 4	2222200000	4322200000	24x0.4	34 Weeks	3	±	0	0	0	0222110000	2100000000	12 Mos. Well	Cure	Markedly hyperactive K.K. have disappeared
Kx9	4 Wks.	6x0.4	0	1.5 Mos.	14	+	0 4 4	1111100000	2210000000	10x0.4	4 Weeks	10	+	4	4	4	1111100000	2210000000	4 Mos. Well	Worse	
Da	10 Mos.	15x0.3+20 l. M. Hg. elsewhere	1 Mo.	0	18	++	0 0 0	2223222100	5543100000	12x0.4	6 Weeks	6	+	0	0	0	1111000000	2100000000	14 Mos. Well	Cure	
B 19	10 Mos.	6x0.3	0	1.5 Mos.	19	±	4 4 4	1222100000	5543210000	22x0.3	20 Weeks	3	±	0	0	2	1111000000	2100000000	15 Mos. Well	Improved	
Dx5	8 Mos.	12x0.4+8 wks. Hg.	6 Mos.	7 Mos.	27	+++	0 0 0	2233100000	2220000000	6x0.4	16 Weeks	3	0	0	0	0	1222100000	2100000000	13 Mos. Well	Cure	
D 15	13 Mos.	12x0.4+8 wks. Hg. pills 3x0.4 7x0.4	6 Mos.	23 Mos.	40	+	0 2 1	1122000000	—	1 I.S. 5x0.4 1 I.S.	42 Weeks	1	+	0	0	0	1111100000	—	30 Mos. Well	Cure	

one exception, consisted of alternating courses of arsphenamin and mercury (by inunction), together with large doses of potassium iodide by mouth. The average course of arsphenamin consisted of from 8 to 12 weekly injections instead of the usual six; the interim between courses was from 1 to 4 months, and was occupied by the constant administration of mercury. In only one or two instances were the two drugs used together. Intraspinal treatment was employed in only one case (D 15).

From a study of these results, it is apparent that patients in this group may be maintained clinically well over long periods of time, and that in the majority of instances the blood and spinal fluid serology may be reduced to normal and maintained there by the use of arsphenamin and mercury by the usual routes of administration, without the use of intraspinal therapy. We have so far not encountered a single patient in this group with whom such a result could not be obtained, provided treatment were sufficiently prolonged. It is, of course, probable that more resistant cases will be met with; in that event, if a six months' trial of routine treatment brought about no improvement, we should at once resort to intraspinal treatment as an adjunct.

The cases in Group III present an entirely different picture. The results in 8 cases of this group are presented in Table XVII. Two patients, both of whom were completely asymptomatic at the time of the first spinal fluid examination, have developed clinical neurosyphilis since that date, and in spite of energetic treatment following the discovery of fluid abnormalities. One of these patients

is now, five years after his infection, and in spite of 57 intravenous injections of arsphenamin, 11 intraspinal treatments, and a great deal of mercury, a typical dilapidated parietic. In both of these instances, however, a long period of time, punctuated by frequent lapses in treatment, had elapsed since the date of infection. The remainder are clinically well. No patients are serologically negative, though on the average much more treatment has been given than in the Group II cases. Three are moderately improved; two slightly improved, two unchanged; and in one the cyto-biology of the fluid is worse after 33 intravenous arsphenamins than before. Intraspinal treatments were employed in three of these patients, resulting in a marked improvement in two.

The contrast between the results in Group II and in Group III cases is strikingly shown in Table XVIII. Of the former, 76.9 per cent of the patients are clinically and serologically well, as compared to none of the latter. This part of the study offers confirmatory evidence of the interpretation given for Chart II, in regard to the effect of the amount of treatment given on the incidence of the various groups, since in both places it is shown that Group II is readily influenced by treatment, and Group III very little if at all.

These results can be applied to the controversy over intraspinal treatment, and our attitude in regard to it, so far as this type of case is concerned, can be briefly summed up. For cases of the type of Groups I or II, intraspinal treatment is usually not necessary, and, if applied at all, should not be used until the patient has

TABLE XVII.
The Results of Treatment of Early Asymptomatic Neurosyphilis—Group III.

Case No.	Duration of disease on admission	Treatment before first spinal puncture.	Lapse in treatment	Interval admission to spinal puncture	First C. S. F. results					Treatment after first puncture		Last C. S. F. results					Time elapsed since first puncture	Clinical result	Serologic result				
					Cells	Glob.	W. a. R.			Gold	Mastic	Asphen-amin	Mercury	Cells	Glob.	W. a. R.				Gold	Mastic		
							0.2	0.4	1.0							0.2						0.4	1.0
11.	8 Mos.	2x0.3	8.5 Mos.	9 Mos.	32	++++	4	4	4	5555321000	—	16x0.35	14 Weeks	7	++	1	4	4	4320000000	5443321000	20 Mos.	Well	Slightly improved
O 1	11 Mos.	4x0.4 elsewhere	+	—	12	+++	4	4	4	5555431000	—	33x0.4	Practically none	61	++++	4	4	4	5544432200	5555321000	27 Mos.	Well	Worse
X 4	6 Mos.	12x0.35+4 wks. Hg.	6 Mos.	11 Mos.	64	++++	4	4	4	5555431000	—	56x0.4	36 Weeks	1	++	0	2	4	5444330000	3321000000	31 Mos.	Well	Slightly improved
14 1*	8 Mos.	3x7 elsewhere 12x0.3+10 wks. Hg.	+	36 Mos.	98	+++	4	4	4	5555430000	5532100000	22x0.4	10 Weeks	70	++++	4	4	4	5555521000	5543210000	10 Mos.	Deep reflexes increased	Unimproved
Zc 27	3 Mos.	27x0.3 and much irregular Hg.	Frequent	46 Mos.	131	++++	4	4	4	5555554100	—	30x0.4 +11 intra-spinal treatments.	Continuously for 14 Mos.	8	+++	4	4	4	5555321000	—	14 Mos.	Asymptomatic at first puncture developed G. P. under treatment	Unimproved
H	24 Mos.	14x0.4. Irregular Hg.	+	22 Mos.	40	++++	4	4	4	55555421000	—	23x0.3 7 intra-spinal treatments	20 Weeks	2	±	0	0	4	1110000000	—	24 Mos.	Well	Improved
Em.	11 Mos.	1x7 elsewhere 15x0.3+12 wks. Hg.	+	10 Mos.	156	+++	4	4	4	5555520000	—	67x0.3	80 Weeks	?	++	3	4	4	5511100000	—	36 Mos.	Well	Slightly improved
S 32	3 Mos.	12x0.4+8 wks. Hg.	0	5 Mos.	65	+++	4	4	4	5554210000	—	5 intra-spinal	—	12	++	0	2	4	5555321100	—	3 Mos.	Well	Improved

TABLE XVIII.

Summary of Treatment of Early Asymptomatic Neurosyphilis, Groups II and III.

	Total cases treated	Lost track of	Clinically well	Developed clinical neurosyphilis	Serologic result			
					Well	Improved	No change	Worse
Group II	13	1	12	0	10 (76.9%)	1	— (23.1%)	2
Group III	9	1	6	2	0	5	3 (100%)	1

had a thorough trial of at least six months of routine anti-syphilitic treatment. For the Group III type, it has been shown that routine treatment accomplishes little or nothing. In these cases intraspinal treatment as an adjunct to routine therapy is of value, because it offers almost the only hope of attaining a serologic cure, and because it tends to bring about improvement more rapidly than the routine method alone, thus obviating some of the dangers attending the too prolonged use of arsphenamin³⁵ and mercury. In a later paper of this series, which will deal with the question of late asymptomatic neurosyphilis, we hope to support our contentions in this respect with additional evidence.

The Prognosis in Early Asymptomatic Neurosyphilis.—It has already been suggested that there is probably no hard and fast line to be drawn between our three groups, and that it may be possible for a patient who falls into Group I on early puncture to belong to Groups II or III if puncture had been carried out later. This change in grouping may also be brought about by the factor of insufficient treatment.

It has occurred to us, as well as to others, that it might be possible for the spinal fluid abnormalities of these groups to clear up spontaneously without the influence of treatment, or that patients showing these changes might remain clinically well without developing neurosyphilis.³⁶ If these two points could be demonstrated, the stress which we lay on early fluid changes would lose much of its emphasis. So far as the spontaneous disappearance of cyto-biologic abnormalities is concerned, we have not had the temerity to allow such patients to

go untreated; and we can say only that, on repuncture of those patients who had voluntarily lapsed in treatment, we have never found a negative fluid. On the contrary, in our few examples of this kind, the later puncture has always shown changes of an intenser degree than the first. We should be inclined to admit the possibility of this conception only in the Group I cases, and we should be very diffident of expression even here.

It is not surprising that adequate statistical data regarding the development of clinical neurosyphilis in these asymptomatic patients are lacking in the literature. Only a few years have passed since the importance of early spinal fluid examinations has been appreciated, and in order to provide data of any value, these patients must be continuously followed over a life time. The longest period of time for which we have been able to follow any untreated case is five years. This patient, a Group III case, is apparently well at present, though she refuses to submit to re-examination. So far, no Group I case, treated or untreated, has developed clinical neurosyphilis. Of our 37 Group II cases, one patient (Sy) whose neurologic examination was completely negative at the time of her first puncture, lapsed in treatment for five months. On her return, her pupils which had been normal were now widely dilated, unequal, markedly irregular, and reacted to light only very sluggishly. One Group II patient is dead (lobar pneumonia). The others, so far as is known, remain well. Those with minor neurologic signs have not gotten worse; those without signs have not developed them.

Of the 17 Group III patients, three have developed general paresis, one after a great deal of treatment, and two after long lapses. A fourth patient has developed outspoken neurologic signs which would now easily allow a diagnosis of cerebrospinal syphilis, though no mental changes are evident. The remainder are apparently well.

The Prophylaxis of Early Asymptomatic Neurosyphilis.—The evidence regarding the origin of these various types of asymptomatic neurosyphilis may be applied to the formulation of a scheme of treatment for early syphilis calculated to reduce the incidence of neurologic damage. As long as the treatment of the early stages of the disease continued to rest largely in the hands of the general practitioner rather than of the syphilologist, we consider it unnecessary to argue that there should be a fairly rigid outline of treatment applicable to the majority of uncomplicated cases. The attempt to individualize treatment will be productive of far more harm than good.³⁶ It is not our intention to enter into the details of our plan of treatment of early syphilis, or the factors which led to its adoption, since this will be fully considered in a later communication.³⁷ It is necessary, however, to point out briefly the essential features of an adequate plan and to indicate the arguments in its favor provided by the data of this paper.

³⁵ Moore, J. E. and Keidel, A.: Dermatitis and Allied Reactions following the Arsenical Treatment of Syphilis. Arch. Int. Med., XXVII, 716, (June) 1921.

³⁶ Kalsiki, D. J. and Strauss, I.: The Significance of Biologic Reactions in Syphilis of the Central Nervous System, with Notes on Treatment, especially Intraspinal. Arch. Neur. and Psych., VII, 98, (Jan.) 1922.

³⁷ Keidel, A., and Moore, J. E.: The Treatment of Primary and Secondary Syphilis. (To be published.)

Anti-syphilitic treatment in early syphilis should be continuous rather than intermittent. There should be no periods of rest between courses of treatment, because of the tendency of lapsed (or irregular) treatment to increase the incidence of spinal fluid abnormalities. In order to accomplish this, arsphenamin and mercury must be used separately. It is probably best to allow a little overlapping, so that the use of mercury is begun just before the last injection of a course of arsphenamin and is kept up until just after the first injection of the next course.³⁸ There are additional cogent reasons for this method of use of the two drugs, a discussion of which is out of place in this paper.

The most indefinite part of any plan of treatment is the length of time for which it should be continued. Our own routine, at first adopted arbitrarily but now justified by the data of Table X and Chart II, has been to treat early syphilis continuously until completely negative serology of the blood and spinal fluid has been obtained, and for one full year thereafter. While it is true that some patients will be cured with less than this amount of treatment, it is equally obvious that others will not be cured; and there is at present no means of an early separation of the two groups. The only safe plan, therefore, is to treat all patients up to the point of cure of the majority.

In early syphilis, treatment is begun with arsphenamin, the first course being of eight doses and succeeding courses of six doses each. Mercury by inunction is started just before the last dose of each arsphenamin course, and continued until the first dose of the next. The interval between arsphenamin courses is at first short (four weeks), and is gradually lengthened with each course. The longest interval permitted is twelve weeks. Spinal puncture is performed as a routine after the first or second courses of arsphenamin. If the standard of serologic cure mentioned above is adhered to, the treatment of sero-negative primary syphilis will usually last exactly one year, and will consist of at least 4 courses (26 doses) of arsphenamin and 26 weeks of mercury. In sero-positive primary and in secondary syphilis, the length of treatment in the average case is usually 15 months (5 courses of arsphenamin and 32 weeks of mercury). If spinal fluid abnormalities are found at the time of the first puncture, this plan is modified according to the group of abnormality present. When treatment has been completed, there ensues a year of probation, during which there must be no symptoms, no positive and at least six negative Wassermann tests (ice-box); and, at the end of the year, negative physical and neurologic examination, negative X-ray of the cardio-vascular stripe, and negative examination of the spinal fluid.

If this plan of treatment could be applied to every case of primary and secondary syphilis, the incidence of asymptomatic neurosyphilis, and in all probability that of clinical neurosyphilis, might be reduced to an absolute minimum. Judging from the data supplied by this paper, the present level of 20 to 25 per cent of clinical neurosyphilis might be reduced to 5 per cent or less. The economic saving to the state, to mention only one of the advantages of this reduction, would be enormous.

SUMMARY AND CONCLUSIONS

1. It has been shown that early invasion of the central nervous system in syphilis, is common, occurring in 26.4 per cent of a series of 352 patients with primary and secondary syphilis. Of 94 early neurosyphilitics, 72 were asymptomatic, and were detected only by the routine application of spinal puncture.

2. Early asymptomatic neurosyphilis may be divided into three sub-groups on the basis of the spinal fluid findings and the response of the various groups to treatment.

3. Invasion of the central nervous system probably occurs in the majority of all patients with syphilis, and unless the course of the disease is influenced from without (by treatment), this invasion takes place in most instances within the first year after infection. The ability of the invading organisms to produce clinical neurosyphilis probably depends on the defense mechanism of the individual patient. The experimental and clinical evidence bearing on these points are reviewed.

4. Early asymptomatic neurosyphilis is more common in the white race than in negroes, but is equally frequent in men and women, of either or both races.

5. Prolonged regular treatment influences favorably the incidence of early asymptomatic neurosyphilis. Irregular or lapsing treatment, on the other hand, markedly increases its incidence.

6. A study of this material from the standpoint of strains of *Treponema pallidum* furnishes no support to the theory of the existence of a neurotropic strain of organism.

7. That the spinal fluid abnormalities of early asymptomatic neurosyphilis are evidence of actual anatomical damage to the nervous system, is indicated by the frequency of certain minor subjective and objective neurologic signs in this class of patients. An appreciation of these signs, and of the significance of a persistently positive blood Wassermann reaction in treated patients, furnishes a clinical diagnostic aid for the recognition of neurologic invasion.

8. Spinal puncture is an indispensable routine procedure in the management of early syphilis. Unless it is employed, many patients will be discharged as cured who are nevertheless candidates for clinical neurosyphilis. It should be performed as a routine after the first or second course of arsphenamin, and unless a lapse in treatment

³⁸ Stokes, J. H.: The Applications and Limitations of the Arsphenamins in Therapeutics. Arch. Derm. and Syph., II, 303, (Sept.) 1920.

occurs, need not be repeated (if negative) until the end of treatment and the probation period.

9. All three groups of asymptomatic neurosyphilis may be serologically and clinically "cured" by appropriate methods of treatment.

10. Early asymptomatic neurosyphilis is the forerunner of clinical neurosyphilis. One patient of our second

group has developed clinical cerebrospinal syphilis. Of the third group, three patients have developed general paresis and one cerebrospinal syphilis.

11. There is suggested an outline of treatment for early syphilis, the uniform application of which will markedly reduce the incidence of asymptomatic neurosyphilis, and probably, therefore, of clinical neurosyphilis.

THE BIOCHEMISTRY OF TUBERCULOSIS*

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In the biochemistry* of an infectious disease two series of metabolism are naturally concerned, that of the invading parasite, and that of the host as modified by the former's action. The manifestations of disease may be looked upon as alterations in the physiology of the host initiated by the metabolism of entering bacteria, although sometimes no more than initiated, since the bacterial growth may serve to fire a train of subsequent events largely non-specific in character.

There is a natural tendency to think of bacterial cells as simple forms of life, and to a certain extent they are, being largely independent of each other, devoid of that correlation so conspicuous in the physiology of higher forms. But, cell for cell, their life processes are probably fully as complicated as those of a liver or kidney cell, or perhaps even one of the central nervous system. They digest their food, build up complex substances from simple ones, they respire, that is to say, take up oxygen and give off carbon dioxide, they excrete waste products, and they reproduce. And if they are shut off from an adequate supply of nutrition, like other cells they die and tend to autolyze.

Leaving out of present consideration those remarkable forms which snatch nitrogen out of the atmosphere, heap up mountains of sulphur, or spread vast marshes with iron, we have an almost endless variety of microorganisms whose methods of obtaining energy and manner of excreting waste are closely akin to those of cells of highly organized life. Some of these are very adaptable, wonderfully equipped foragers, not overly particular, within reasonable limits, about the temperature at which they work or the form in which the great classes of food-stuffs are supplied to them. They grow in relatively large masses, many dying that others may live, and in their process of dissolution many kinds of them liberate powerful ferments which make more food available for the rest. In view of their relation to organic decay they have received the name of "saprophytes." Etymologically they are decay-growers. They live their day, grow, die

and decay themselves, and by virtue of their involuntary sacrifice serve the high purpose of maintaining those great cycles of the elements in which a certain amount of carbon, nitrogen and sulphur is continually available in a mobile state suitable for the nutrition of other forms.

Not all bacteria by any means are so useful as these great cycle maintainers. Not a few, evolutionary accidents in the mighty, invisible struggle for the survival of the fittest, must eke out a more or less miserable existence, dependent for their nurture largely upon the unwitting, lavish generosity of the great hosts of proteo-, lipo- and cellulo-clastic forms, or upon the less willingly provided maintenance of more highly organized life.

Of this last set a certain number have acquired what amounts in the end to a protective adaptation, the mysterious condition of parasitism. This mode of existence, the factors of which were so clearly defined in a recent address by Theobald Smith, has for its essential elements the ability on the part of invading microorganisms to multiply within the tissues of a living being, and to multiply in such place and manner that an easy exit is afforded, from which some of the invaders may escape to another host of the same sort. Thus parasitism as a permanent mode of life necessarily involves a cycle including more than the mere ability to multiply in tissues.

It is, however, only to the first of these factors of parasitism that I shall now make reference, limiting my remarks to the special case of multiplication within the tissues of the human host. The remarkable thing is not that there are so many organisms that possess this capacity, but that there are so few, and that those bacteria which have adapted their nutritional requirements to the difficult conditions obtaining within the host, should be so helpless in the region of bounteous plenty outside, while those which attack dead organic material so energetically seldom establish a home beyond the first barrier of living cells. The typhoid bacillus pursuing its increasingly lonely way down densely populated sewer pipes is a pitiful object beside the infinitely

*Read before the Lannec Society of The Johns Hopkins Hospital, November 21, 1921.

more vigorous proteus family; so poorly equipped for the journey is it that after five or six days few members of its group survive to tell of a different phase of their existence, the tale of deadly destruction wrought so short a time before in an environment impregnable to the surrounding billions of saprophytes.

Among these bacteria which, so far as I can see, serve no useful purpose in the great scheme of life, is a group of organisms characterized by the protoplasmic possession of a large amount of non-saponifiable lipoid, which so modifies their reaction to some of our chemical reagents that they can be readily picked out from the rest by a simple application of appropriate stains. This is the acid-fast group. Within it are several varieties, including parasites adapted to life in different hosts, namely, the tubercle bacilli, and saprophytes unable to maintain such existence, although flourishing more or less widely outside.

Time does not permit us to puzzle long over the evolutionary relationship of these forms, but that there is a relationship, remote though it may be, is at least suggested by certain facts. The most outstanding of these is their interchangeability as antigens in the so-called biological reactions, which indicates, according to the more general concepts of immunology, that they possess in common certain proteins, specific to the degree that they are present in no great concentration, or not at all, in the bodies of other bacteria. The "tuberculin," if I may use the term, formed by the growth of a grass bacillus in bouillon, will elicit a typical reaction in a tuberculous animal, as the similar product from a hay bacillus will not, and there seem to be more investigators who believe in a common "acid-fast fixation of complement" than who do not. This refers to qualitative conditions and does not mean that the organisms of the group may not be separated by biologic tests made on a careful quantitative basis.

Certainly these organisms are closely enough allied in their chemical makeup so that it would be difficult or almost impossible to distinguish them as dead chemical material. Give a man an autoclaved culture of grass bacilli and one of tubercle bacilli and he would be a keen analyst if he could distinguish between them. He might filter them off and stain them, noting that they were resistant to acid decolorization, and he might extract them with chloroform and find a content of waxy substances in the neighborhood of 30 per cent, and be able to say, "These are not hay bacilli or typhoid germs; they belong to the acid-fast group." He might go further and make emulsions of the dead bacteria and test them out against tuberculous guinea-pigs by skin inoculation, and confirm his previous conviction on the group to which the organism belonged, and he might perhaps notice that one of his preparations brought out a distinct reaction in

quantities definitely less than those required with the other, but he would be fortunate if he could be sure.

It is only with the living material that he could stand up without fear and say, "These are tubercle bacilli, and these are something else in the acid-fast group." And he would have one or perhaps two lines of fire on which he could hit his mark. The first of these would be the establishment of the condition of parasitism in an appropriate host. The second would be the proof of a characteristically different metabolism in non-living culture media. Of these the first would be a bull's-eye, made on a shot through the dark. The second would be made through the light, in so far as known chemical reagents are clear to our understanding, as a guinea-pig is not, but it would probably not be a bull's-eye—at present at least. I am convinced that a time will come when a relation of virulence to metabolic activity will be established, but little progress has been made to date. So far as I know, the surest chemical criterion at present for a tubercle bacillus is that discovered by Nocard thirty-five years ago, its marked glycerophilism. Our hypothetical solver of bacteriological unknowns could, I believe, find that for one of his strains glycerol was a practical necessity, while for the other ethyl alcohol or the neutral salt of lactic or pyruvic acid would serve equally well. Or he might make up a medium of neutral reaction containing phosphoric acid and certain inorganic salts, with a single amino acid like leucine or alanine as the sole organic material, and find that the grass bacillus grew readily on this, whereas the tubercle bacillus did not. In either case he would be dealing with a distinction in carbon metabolism.

If he attempted to distinguish them on the basis of nitrogen metabolism, he would be working on a more difficult problem, for he would find that, if hard enough pressed, either one could satisfy its nitrogen requirement with ammonia alone or with the nitrogen of simple amino acids or acid amides, as alanine or propionamide. He might be able to make some distinction on the readiness with which they abstracted that ammonia from different nitrogenous combinations, however, although he has little enough information to go on at present. For instance, in their action on amines, certain differences might be noted, inasmuch as some of the grass bacilli have been found to deaminize ethyl amine. The tubercle bacillus as well as a good many of the saprophytic acid fasts do not. Again, in their utilization of imino nitrogen, as seen in the compound creatinine, a more marked difference is shown, as this nitrogen seems unavailable for the tubercle bacillus, while a larger number of the saprophytes, including several strains of grass and smegma bacilli, in my experience, are able to grow on this compound as sole source of nitrogen, presumably hydrolyzing it and thus securing amino nitrogen. Also he might find that certain combinations of nitrogen and carbon,

while not necessary, were favorable. Indications have been found that the diamino acids and tryptophane favor growth, just as they do in animal life, perhaps because they are a necessary part of bacterial protein molecules, and their synthesis is a matter of some difficulty. That both organisms possess the capacity for synthesizing the aromatic rings, however, if forced to do so, may be readily proved by growing them with an ammonium salt as the sole source of nitrogen and testing the product some generations removed, for tryptophane, tyrosine and purine bases. The tests would be positive.

All in all, much is yet to be done to furnish an adequate basis for correlating metabolism and virulence. Some of the lines are so open, and so obviously important, that I feel like apologizing for coming before you tonight, as a worker in the field, to report that some of the most promising have been scarcely touched. For instance, to mention just one, much of natural resistance of the tubercle bacillus to digestion *intra vitam* has been attributed to its high wax content. But the avirulent acid-fast have a high wax content too—at least the few of them so far examined. What is the difference?*

So far we have considered chiefly intake and synthesis in our brief reflection on the metabolism of the tubercle bacillus. The other side, catabolism and excretion, is at least as important from our point of view. As the tubercle bacillus grows in a bouillon culture, or in the disintegrated tissue of a caseous tubercle, diffusible molecules of the medium must be passing through its semipermeable tissue all the time, there to meet certain enzymes which knock off various parts of the molecule; some of which are retained by the cell, caught on the fly so to speak, while others diffuse back into the medium. The chief blocks so chipped off are ammonia, various acids from the amino acids, and oxidation products of the carbohydrates. The variation in the amounts cast out leads to those more or less characteristic changes in reaction depicted in the Theobald Smith curve.

Now these simple substances are not the only materials getting out of the bacilli into the medium. For the medium of growth, freed from bacilli, now possesses certain properties not conferred by the products of metabolism just mentioned. It contains something which is poisonous to a tuberculous animal. Inasmuch as the symptoms elicited by this broth are the same as those called forth by any sort of watery extract of tubercle bacilli, carefully washed though they be from adhering broth, it may be assumed with some confidence that it is some product of the bacteria in the broth, and not the reverse, which causes the reaction. Now what of their own substance have the bacilli contributed to the sur-

rounding medium in the weeks or months of their growth? A not unnatural supposition is that some have died and autolyzed, furnishing a certain amount of the products of protein autolysis. Somewhat against this hypothesis is the well known fact that tubercle bacilli do not autolyze to any great extent. Still a very little might be produced of a very potent substance.

That tuberculin is not a product formed extracellularly by action on the constituents of the medium, is practically certain, inasmuch as strong tuberculin can be made from simple media, like ammonium phosphate plus glycerol. In fact such media furnish the best material for a study of the nature of tuberculin, since there are no complex interfering substances. Now if tuberculin is protein in nature, like other materials concerned in immunity, it seems as if such media should be ideal for proving that fact. And yet just here there is the most amazing discrepancy of results. A half a dozen men, including Lockemann, report growing tubercle bacilli on such media, filtering them off cleanly, obtaining a true tuberculin with the material, and precipitating by ammonium sulphate or alcohol a substance in it which gave all the protein reactions, and was itself active as tuberculin. And a half a dozen other men, including Löwenstein and Pick, get no such thing. They get an active tuberculin, to be sure, but it gives no protein reactions. As far as my own experience goes, I have twice made tuberculins, once by growth on an ammonium chloride-glycerol medium, and once on an alanine-glycerol medium, which were active on tuberculous guinea-pigs, but gave no protein reactions, not even the simple biuret test.

In favor of the view that it is protein in nature, are the experiments of a number of investigators who have weakened or destroyed tuberculin by the action of such proteolytic ferments as pepsin, trypsin and erepsin. Perhaps, when the problem is solved, it will be found that tuberculin is a polypeptid, an autolytic product of the tubercle bacillus, protein in its nature in that it is derived from protein, but not sufficiently complicated in its structure to give the usual protein tests. This finding would force us to modify some of the prevailing concepts of immunity. Certainly an attractive problem here awaits solution.

One more word before I pass to the subject of the metabolism of the infected host. That concerns the conditions under which bacteria, even parasites, may multiply within a living body. The researches of Rettger and his associates have pretty definitely brought out the fact that bacteria are unable to grow on pure proteins or coagulated proteins, or in many instances even on pure proteoses or peptone. They need a certain amount of still simpler products of protein digestion from which to construct their enzymic machinery for the cleavage of larger molecules. This is a significant point in their

* It has been learned, since this address was given, that while the total quantity of lipin in tubercle bacilli and saprophytic acid-fast bacilli is about the same, the proportion of that lipin as wax is much higher in the tubercle bacillus.

relation to live cells. Living protoplasm contains no great amount of the simple molecules which are favorable to bacterial growth; consequently, living tissues are a poor feeding ground for germs. Once these tissues die, however, or, in other words, once the rate of catabolism greatly exceeds anabolism, or the latter ceases altogether, autolytic products accumulate, and the conditions for bacterial multiplication are at once established. This point will be referred to again when we come to consider the relation of the growth of the tubercle bacillus to necrosis. Doubtless many other important factors are concerned, such as the oxygen and carbon dioxide tensions, as has been so well brought out in the work of Wherry and Ervin and in the very interesting recent investigation by Corper. Death of the tissues probably plays a rôle here too.

So much for the present concerning bacterial metabolism.

A discussion of the other side of the picture, namely, the altered physiology of the host, properly begins with a consideration of the most characteristic lesion of tuberculosis, caseation. In this type of necrosis, tissue becomes pale grey or yellowish in color, with a certain resemblance to cheese, which is indeed closer from the point of view of chemical composition than that of appearance to the eye, the necrotic mass consisting, like cheese, essentially of coagulated protein and finely divided fat. The protein is chiefly simple protein, not nucleo—or otherwise conjugated. In fact the lessened amount of nuclear material, such as phosphoric acid and purine bases, is one of the striking chemical differences between caseous and normal tissues. Autolysis, which is on an extremely low plane, has at least gone far enough to bring these nuclear components into diffusible form. The total amount of protein is about the same as in the corresponding area of normal tissue, as is that of lipins, although certain qualitative differences are to be noted in each group. The proportions of cholesterol and lecithin are altered, and the iodine number, or index of non-saturation of lipins, is changed, but the variations from normal are not so great as to cast doubt on the assumption that the caseous matter consists almost entirely of disintegrated preexisting tissue, modified to a slight extent by slight autolysis and the introduction of a small amount of foreign protein and lipin from the bodies of tubercle bacilli.

The cause of caseation necrosis in tuberculosis is still unsettled. Some investigators have claimed specific coagulating properties on the part of substances in the body of the tubercle bacillus. Auclair years ago reported producing caseation with a certain fraction of the fatty material. Morse makes a similar report today. Ruppel obtained a protamine, which he called "tuberculosamine," which like other protamines caused a precipitation of proteins from their solutions. Others have considered

caseation as allied to the Arthus phenomenon, the result of the lodgment of a foreign protein on highly sensitized soil. In this case we are perhaps to think of cleavage of the foreign protein with the production of a toxic, coagulating fraction. It seems to me, however, that too much emphasis cannot be laid upon the unquestioned condition of local anemia as a causative factor in necrosis. The mechanism of its production offers room for argument, although several plausible explanations are readily thought of. The entering bacillus may produce substances causing an initially minute amount of capillary thrombosis, enough to produce necrotic material capable of giving sustenance to the bacilli present. However, the anemia may be more simply explained. Every entering mass of tubercle bacilli is promptly met by a perfectly non-specific foreign body reaction, the proliferation of a fixed phagocyte, the so-called epithelioid cell. The mere multiplication of these cells in a given area must be sufficient, through the pressure exerted, to shut off some of the capillary circulation, as Mallory explains necrosis in typhoid fever. Thus a local anemia may be readily established, with subsequent death of the cells of the anemic area. Then, and not until then, the conditions for bacterial multiplication are established, the bacilli obtaining the nutriment which they were unable to obtain from living cells from the autolytic products of the dead ones. After this start bacilli are transported, one way or another, to neighboring areas and an endless chain of foreign body reaction, anemic necrosis, and bacillary proliferation established.

A more difficult problem is to explain the long continued chemical *status quo* of the caseous tissue. In some way this must be dependent upon the presence of the tubercle bacillus. The like is not often seen in other forms of necrosis. An anemic infarct shrinks as its substance is slowly absorbed and replaced by entering fibroblasts. A cancerous nodule becomes umbilicated as its central anemic mass degenerates and diffuses away. But in the tubercle, the fibroblasts, although they may ring the lesion in leathery form, have little tendency to enter on radiating lines, while solution of the central, morphologically disintegrated tissue by autoenzymes is conspicuous by its absence. The lack of chemotaxis for motile cells is perhaps explained by the failure of the cellular enzymes to liberate diffusible products. Certainly extractives are present in but minute amount. Even in extreme cases of fluidity, as in cold abscesses, the crystalline products of the autodigestion of proteins are almost absent. Nor can proteases be demonstrated by the Müller-Jochmann plate method. Why this fundamental failure of autolysis? Have the autolytic enzymes been destroyed, or are they merely held in a state of inactivity? Jobling and Peterson have furnished some interesting information on this point. They noted first, that proteolytic action in general, and autolysis in particular, were inhib-

ited by the soaps of unsaturated fatty acids, while not affected by the saturated ones; secondly, that caseous areas, which characteristically did not autolyze rapidly, contained such soaps in appreciable amount; and thirdly, that the saturation of these soaps with iodine removed their antiproteolytic action, following which autolysis pursued a normal course. Finally, they were able to demonstrate the presence in the tubercle bacillus itself of unsaturated fatty acids, the soaps of which are more active in proportion to their iodine value in inhibiting trypsin and leucoprotease than soaps of linseed and olive oil, and that these soaps too can be deprived of their inhibitory action by iodine. Their conclusion naturally was that the unsaturated fatty acids of the tubercle bacillus are a very important factor in the failure of absorption of caseous tissue. I have always felt that this piece of work is very much in need of amplification. For instance, it would be desirable to know if the leprosy bacillus, which excites a foreign body reaction without cessation, is devoid of these antiproteolytic properties.

We may next consider this caseous mass for its bearing upon the physiology and metabolism of the host. It has its specific and non-specific elements, as far as tuberculosis is concerned. In the first place, it contains tubercle bacilli, which, chemically considered, are foreign proteins, proteins which by their continued presence within the body have so sensitized the tissues of that body that they react, more or less violently with hyperemia and exudation, to the same material, whether it arrive at a given point by dissemination from the original focus, or by fresh introduction from without. Yet it is unlikely that this reaction to "tuberculin" accounts for all, or even the greater part of the toxemia of tuberculosis. For, secondly, the tissue of the caseated area, once homologous, before its coagulation, has now become a foreign protein too. Whether the body is allergic toward it, as it is to tuberculo-protein, is a question. Be that as it may, the substance is appreciably toxic *per se*. The toxicity of the products of incomplete protein digestion, proteoses and peptones, is too well known to require comment. But is much of such diffusible substance present in the caseous tubercle? We have noted before that self-digestion of the caseous mass occurs on but a small scale.

In this connection may we not refer to a very instructive set of experiments by Dr. Krause? You are doubtless quite familiar with these; yet they seem to me so important for our conception of the chemistry of the disease that I cannot refrain from repeating their story in some detail. Krause started out with the idea that foci at the height of reaction probably contained more toxic material than non-reacting foci. Accordingly, to prove this he gave large doses of tuberculin to tuberculous guinea-pigs, killed them off during the acute stage

of the subsequent illness, and found, as he knew he would, that the tuberculous foci were in a condition of inflammatory reaction. He then emulsified these inflamed foci in physiological saline, centrifuging the product to remove masses of a size sufficient to act as emboli, and injected the clear juice intravenously into normal animals. He found, as he anticipated, that the material was highly poisonous. Then he went a step further. He injected emulsions similarly prepared from the non-reacting foci of animals which had never received tuberculin. Somewhat to his surprise, I believe, he found that such emulsions too were markedly toxic, as much so as the first ones. He next took tissue from healthy guinea-pigs, emulsifying and injecting normal organs, and found that this material too was toxic for normal animals, and furthermore, just as much so as that from a tuberculous focus. If any of these emulsions were filtered, however, through a Berkefeld candle, the product was no longer toxic. These experiments indicated to Dr. Krause that an animal is poisoned by any kind of cellular material which gets into its blood stream in large amount in unit time. An abundance of confirmation of these experiments on the effects of tissue juice in the blood stream of normal animals has accumulated in the literature of fields other than tuberculosis. The recent experiments of Karsner and Hanzlik and of Mills are instructive. It is especially interesting that lung tissue furnishes exceptionally toxic substances. All these investigations prove, I believe, that a storehouse of disintegrating protein, even though inhibited in its autolysis to such an extent that no great amounts of toxic proteose and peptone are absorbed, is yet a source of poison to the organism containing it. On the periphery of each caseous mass in the body of a consumptive a certain amount of this material must be all the time being swept into the circulation. Any condition which will increase the circulation at that point naturally will favor absorption. Such a condition occurs in the focal active hyperemia which follows the administration of tuberculin, or the dissemination of tubercle bacilli to fresh but sensitized soil, the latter being a process constantly occurring in progressive tuberculosis. Thus, the toxemia of progressive tuberculosis may be thought of as to a considerable extent non-specific in character, dependent upon the absorption of disintegrating protein, but it must be continually borne in mind that it is aided, in the manner noted, by one which is quite specific in nature, the focal inflammatory reaction to tuberculo-protein.

Let us now take up the alterations in the physiology of the host, dependent upon absorption from his focus, regardless of the site of the latter, so long as it is in a sufficiently unprotected state, leaving out of consideration those changes in metabolism which depend upon the location of the lesion, such as the diminished gas exchange following obliteration of lung volume, or dis-

turbed absorption resulting from intestinal ulceration, which also have sequelæ of abundant chemical interest.

One of the most striking clinical manifestations of this absorption is fever. And in a perfectly non-specific manner to a certain extent changes are produced in the patient's metabolism, not because he has tuberculosis, but because he has fever. This is hardly the place for a discussion as to the cause of the fever in tuberculosis, but we may simply recall the fact that, since the time of Traube, fever has been attributed to a diminution in the ability to discharge the heat produced, the regulatory control being lost through the action of some poison upon the vasomotor centers, and we may state further that according to modern opinion, developed largely from the work of Vaughan, that poison is frequently protein in nature. Indeed there is much ground for believing that the fever in tuberculosis is, like the fatality in the case of Dr. Krause's guinea-pigs, due to the presence, in the blood stream, of foreign protein or protein cleavage products, and that these in turn have two sources, tuberculo-protein and the protein of the disintegrating caseous mass, of which two the latter is by far in excess.

Whatever be the mechanism involved in the production of fever, once fever is established, metabolism may be modified more or less profoundly as a result of it. "Warmed tissue metabolizes more material than cooled tissue," writes Lusk, citing the classic experiments of Pflüger, who curarized rabbits, thus paralyzing their voluntary muscles and capacity for increased work, at the same time applying external heat, and noted that as the rectal temperature rose from 39° to 41° the oxygen absorption increased 10 per cent for each degree. Plenty of confirmation has appeared since the time of Pflüger, and a large literature has accumulated on the nature of the material metabolized. That basal metabolism is increased in tuberculosis has recently been shown by Grafe, who found in twenty patients a raising of heat production per kilogram varying between 10 and 80 per cent, the higher figures being for febrile cases. Whether such metabolism can be considered "basal" is a technical question discussed by McCann and Barr, who also note an increased metabolism in fever, which at 40° may be 30 per cent above normal.

The effect of fever upon protein metabolism and nitrogen elimination is more complicated. One of the most interesting investigations on the subject was that of F. Voit, who found that artificially raising the temperature of a fasting dog to 40-41° for twelve hours increased the nitrogen elimination 37 per cent above normal. That the action was not a selective one upon protein, however, was indicated by the fact that a preliminary feeding of 30-40 grams of sugar prevented this increase in protein metabolism, which was thus seen to be simply a part of the general increase, occurring only when the store of more readily burnable glycogen was depleted. That a

loss of nitrogen occurs in infectious fevers has long been known, and that this is greatest during the height of fever is also generally true.

How much of it, however, is due to the fever and how much to more direct action of the absorbed material which causes the fever, remains to be determined. Many writers speak of a toxic destruction of protein. May, for instance, says, in the conclusion of his comprehensive review of the subject of metabolism in tuberculosis: "The wasting of tuberculous subjects is due primarily to poisons absorbed from tuberculous nodules. Large doses cause a direct injury to cell protoplasm. The amount of protein destruction attributable to this cause is not very large, but becomes of importance when continued for a long period of time and when there is no compensatory regeneration. It appears that the power to regenerate on the part of these cells injured by toxins is greatly reduced and in severe cases entirely lost." He goes on to write, however, "Higher degrees of toxic action are usually accompanied by fever, and the metabolism of the febrile consumptive follows the same laws as metabolism in the fever of other causes. There is an increased protein destruction, but not an increased fat metabolism, except as may incidentally result from dyspnea, chill or muscular activity."

Graham and Poulton, in Friedrich Müller's clinic, have showed that in man a body temperature of 40.2°, brought about through the influence of a steam bath, does not of itself cause an increase in the metabolism of protein. In their carefully controlled experiments the abnormally high body temperature was maintained for several hours and yet there was never any increase in the breakdown of body protein as the result of hyperthermia. Coleman and Dubois, in their investigation on the metabolism in typhoid fever, found it impossible to escape the conclusion that the destruction of protein was caused by the toxins of the disease, noting that in some cases protein destruction continued for several days after body temperature had reached a low level. In their recent work on tuberculosis McCann and Barr adopt a similar point of view concerning the cause of protein destruction, and find that in this disease the toxic destruction is conspicuously less than in typhoid fever. An interesting finding in tuberculous patients was normality in the specific dynamic action of proteins, indicating that ingested protein was undergoing normal catabolism, and not serving merely as partial replacement of greatly wasted tissue proteins, as seemed to be the case in Coleman and Dubois' typhoid patients, who, probably because of the high level at which protein metabolism was being maintained, failed to show the normal specific dynamic action of ingested protein. That some toxic destruction occurred, however, was shown by their results on the minimum nitrogen excretion of the tuberculous organism, 5-6 grams per day, somewhat more than that which

may be obtained in healthy men, although distinctly less than the lowest obtainable in typhoid fever. I may add that Ahlquist has found that while normal children, four to nine years of age, showed a retention of at least 20 per cent of the nitrogen absorbed, tuberculous children of the same age retained only 8-14 per cent. This is confirmatory experimental evidence for an impression we have long held concerning the growing tuberculous child.

Summarizing the results of the investigations cited we may conclude that in progressive tuberculosis the rate of metabolism is increased, as a result of the operation of two factors, hyperthermia and the toxic effect of a foreign protein upon protoplasm. As far as protein destruction is concerned, the second of these seems to be the more important, for hyperthermia *per se* does not increase nitrogen elimination in the normal subject. Yet it may well be that in the increased general metabolism induced directly by fever, the tissues become impoverished in those protein-sparing constituents which serve to prevent protein destruction at similar temperatures in health, and that protein may for this reason be drawn upon to maintain the high level of total energy transformation.

In less severe, afebrile tuberculosis such tissue destruction as does take place is probably the result of the action of some poison upon the cells. McCann and Barr's results justify the conclusion that in such cases basal metabolism is but little altered, and protein metabolism but slightly above the normal. Even Grafe, who noted an increase in basal metabolism which at times reached 80 per cent, comments on the marked conservation of the

afebrile tuberculous organism for its protein nitrogen.

Going back to reconstruct our chain of chemical factors involved in the progression of tuberculosis we have the tubercle bacillus as the first link, a wax-armored micro-organism, maintaining itself in necrotic tissue, picking and choosing its nutriment from the heterogeneous mass set before it, utilizing the glycerol of hydrolyzed fats, and probably building its wax therefrom, taking ammonia from certain of the amino acids produced in the digestion of dead protein, utilizing others directly to speed up the process of synthesis of its own protein, autolyzing to a slight extent, sufficiently to sensitize the surrounding host to its diffusible protein products, being carried by the lymph, by phagocytes or otherwise, to new soil, there to be met by a non-specific foreign body response, which in the end operates to produce anemia and death of the isolated cells. Then we have the failure of that dead tissue to autolyze, perhaps because of the presence of ferment-inhibiting substances within the bacillus, the phenomenon of caseation. Finally, there is more or less absorption of foreign protein from that focus, that of the bacillus itself and that of the disintegrating tissue, both toxic to the body protoplasm, both capable of causing fever and stimulating the metabolism of the host, so that in severe cases the typical picture of consumption ensues. I feel that this is a very sketchy treatment of a tremendous subject, and that some of the conclusions drawn may prove incorrect, but they may serve to draw attention to the intricate chemical mechanisms involved in the disease.

THE DISSEMINATION OF BACTERIA IN THE UPPER AIR PASSAGES

III. THE RELATION OF BACTERIA TO THE MUCOUS MEMBRANES

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In the preceding papers of this series¹ it was shown that bacteria and foreign particles introduced into the mouth are carried back by definite currents and rapidly eliminated. In certain cases, to be sure, foreign organisms may persist for long periods of time, but in such instances they are usually found in a focus of diseased tissue such as an infected tonsil. The free mucous surfaces of the mouth and tongue, on the other hand, preserve a fairly simple and constant flora and foreign organisms deposited thereon are usually rapidly eliminated. The question, therefore, arises as to what prevents the removal and elimination of the "normal" flora. Why does the tongue not rid itself of its habitual flora just as it does of foreign organisms? It has usually been assumed that the harmless "mouth bacteria" were growing freely in the buccal secretions just as they might in

a test-tube of broth. But the above considerations would seem to indicate that some firmer union must exist between the microorganisms and the mucous membranes of the host. The present communication deals with the nature of this union and its mechanism.

METHODS

The general method of approach was based on the following considerations. If the members of the habitual "normal" flora of a healthy mucous membrane are merely growing freely on that surface in the mucous layer, it should be possible by some process of intensive cleansing to remove a large part or all of such organisms. If bacteria still persist in spite of such procedures, one must assume a very close affinity, both functional and anatomic, between the organisms and the mucous mem-

brane, quite analogous to that which obtains in the case of a foreign pathogen and a focus of diseased tissue.

In the first group of experiments attempts were made to remove the bacterial flora of the tongue by washing with water. The individuals studied were all healthy men with clinically normal buccal cavities. The tongue was the point of attack because it lent itself most readily to the experimental procedure.

EXP. I. Dec. 8, 1921. The mouth was thoroughly rinsed with water and the anterior half of the tongue was then alternately flushed and scrubbed with sterile gauze pledgets for five minutes. Cultures were made before and after the procedure from approximately the same spot on the tongue and they were plated by similar methods on various media. Results of Cultures: (aërobic methods only).

<i>Culture before washing</i>	<i>Culture after washing</i>
(1) ∞ Gram-negative cocci	(1) ∞ Gram-negative cocci
(2) Many M. Tetragenus	(2) Many M. Tetragenus
(3) ∞ non-hemolytic streptococci	(3) ∞ non-hemolytic streptococci
(4) Many hemolytic influenza bacilli	(4) Many hemolytic influenza bacilli

Comment: The cultures showed no qualitative or quantitative change in the flora after the above procedure.

EXP. II. Jan. 3, 1922. The same procedure was employed, but the washing was kept up for fifteen minutes.

<i>Culture before washing</i>	<i>Culture after washing</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) A few Gram-negative cocci	(2) A few Gram-negative cocci
(3) Many grey streptococci	(3) Many grey streptococci
(4) Many green streptococci	(4) Many green streptococci
(5) A few pin-point alpha hemolytic colonies (not identified)	(5) A few pin-point alpha hemolytic colonies (not identified)

Comment: After washing the tongue with water for fifteen minutes there was no qualitative change in the flora. In this experiment there seemed to be a slight quantitative decrease in the number of colonies but it was within the limit of error of swab-culture technique.

EXP. III. Jan. 5, 1922. In this experiment it was decided to push the effect of washing with water to the limit. Three procedures were carried out successively. First the mouth was thoroughly rinsed, then the anterior part of the tongue was scraped with the teeth and finally it was scrubbed with sterile gauze. These three maneuvers were repeated continuously for thirty minutes. At the end of this time the tongue was irrigated with five liters of water. Cultures were made from the same spot on the anterior tongue before the experiment, after the thirty minute period of washing, after thirty-five minutes, and after sixty minutes.

<i>Culture before washing</i>	<i>After 30 minutes</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) ∞ Gram-negative cocci	(2) ∞ Gram-negative cocci
(3) Many green streptococci	(3) Many green streptococci
(4) Many grey streptococci	(4) Many grey streptococci
(5) A few hemolytic influenza bacilli	(5) A few hemolytic influenza bacilli
(6) A few Staph. aureus	

<i>After 35 minutes</i>	<i>After 60 minutes</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) ∞ Gram-negative cocci	(2) ∞ Gram-negative cocci
(3) Many green streptococci	(3) Many green streptococci
(4) Many grey streptococci	(4) Many grey streptococci
(5) A few hemolytic influenza bacilli	(5) A few hemolytic influenza bacilli
(6) A few Staph. aureus	(6) A few Staph. aureus

Comment: After washing for thirty minutes there was no qualitative or quantitative change in the cultures save that Staph. aureus which was present in very small numbers in the control was absent. Inasmuch as it was recovered in the two following controls, this absence in one culture must be an experimental error which is to be expected with the crude swab method where only a few organisms are present.

EXP. IV. Jan. 10, 1922. The mouth was thoroughly rinsed with water and the tongue was then scrubbed with water and a stiff toothbrush with frequent irrigations. At the end of fifteen minutes the scrubbing had progressed to a point where the tongue began to bleed, and the experiment was discontinued. Cultures were made before and after the scrubbing from the same site.

<i>Culture before scrubbing</i>	<i>Culture after scrubbing</i>
(1) Many M. Tetragenus	(1) Many M. Tetragenus
(2) A few hemolytic influenza bacilli	(2) A few hemolytic influenza bacilli
(3) Many Gram-negative cocci	(3) Many Gram-negative cocci
(4) Many green streptococci	(4) Many green streptococci
(5) Many grey streptococci	(5) Many grey streptococci
(6) A few Gram-positive diphtheroids	(6) A few Gram-positive diphtheroids

Comment: There was no demonstrable qualitative or quantitative change in the flora after the above procedure.

DISCUSSION OF EXPERIMENTS I-IV.

It will be seen from the control cultures that the organisms present on the tongue at the start of the experiments were members of the "normal flora"—that is to say, organisms which are present in practically all healthy mouths and which are found widely spread throughout the mucous membranes of tongue, cheeks, tonsils and pharynx—with the exception of the hemolytic influenza bacillus, and the staph. aureus in Experiment III. While some of the bacteria were undoubtedly removed in the washing process, the number was relatively so small that there was no demonstrable change in the appearance of cultures made after the most vigorous sort of a scrubbing process. It becomes clear, then, that the members of the "normal habitual" mouth flora are not simply growing free in the mouth secretions but that they are intimately bound to the mucous membrane just as are foreign organisms in a focus of infection. Their complete removal is apparently no more readily effected than that of diphtheria bacilli, for example, which are breeding in the depths of an infected tonsil. The nature of this adhesion to the mucous membrane will be discussed below, but further attempts at removal by other means will first be described.

The preceding experiments had indicated that the actual breeding place of the mouth flora must be beneath

the layer of mucus and very near to the actual cells of the mucous membranes. Means of drawing the organisms out and making them more accessible to the washing effect of irrigations were considered. It seemed possible that strongly hypertonic solutions might produce the desired result. Sodium chloride was therefore tried.

EXP. V. December 13, 1921. The mouth was washed and the tongue was then scrubbed with saturated salt solution for ten minutes. At the end of this time it was irrigated with two liters of water.

<i>Culture before procedure</i>	<i>Culture after procedure</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) Many Gram-negative cocci	(2) Many Gram-negative cocci
(3) Many non-hemolytic streptococci	(3) Many non-hemolytic streptococci
(4) Many hemolytic influenza bacilli	(4) Many hemolytic influenza bacilli

Comment: No demonstrable qualitative or quantitative change in flora.

EXP. VI. A piece of rubber dam with a window about 3 cm. square was placed on the tongue. This allowed manipulations without contamination of the experimental area by the saliva. A paste of sodium chloride was applied to the tongue. The subject almost immediately experienced a strong burning sensation. After three minutes the salt was washed off and the tongue thoroughly irrigated with water. The salt pack was then reapplied and the above procedures repeated for fifteen minutes. Cultures were made before and at the end of the experiment.

<i>Culture before salt application</i>	<i>Culture after salt application</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) Many Gram-negative cocci	(2) Many Gram-negative cocci
(3) A few hemolytic influenza bacilli	(3) A few hemolytic influenza bacilli
(4) A few green streptococci	(4) A few green streptococci
(5) A few grey streptococci	(5) A few grey streptococci
(6) 1 colony Staph. albus	

Comment: No quantitative or qualitative change made out.

EXP. VII. The tongue was scrubbed and irrigated with a saturated solution of sodium bicarbonate for five minutes.

<i>Culture before bicarbonate</i>	<i>Culture after bicarbonate</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) Many Gram-negative cocci	(2) Many Gram-negative cocci
(3) ∞ non-hemolytic streptococci	(3) ∞ non-hemolytic streptococci
(4) A few Gram-positive diptheroids	(4) A few Gram-positive diptheroids

Comment: No qualitative or quantitative change made out.

EXP. VIII. Similar to Experiment VI, except that a pack of bicarbonate of soda was applied for fifteen minutes.

<i>Culture before procedure</i>	<i>Culture after procedure</i>
(1) ∞ M. Tetragenus	(1) ∞ Tetragenus
(2) Many Gram-negative cocci	(2) Many Gram-negative cocci
(3) A few hemolytic influenza bacilli	(3) A few hemolytic influenza bacilli
(4) A few green streptococci	(4) A few green streptococci
(5) A few grey streptococci	(5) A few grey streptococci

Comment: No qualitative or quantitative change made out.

EXP. IX. It was thought that possibly the adhesive qualities of mucus might have prevented to some extent the removal of organisms by the foregoing procedures. Sodium carbonate

which is a solvent for mucus was therefore tried. The tongue was scrubbed and irrigated for fifteen minutes with two per cent sodium carbonate—the greatest concentration that could be endured.

<i>Culture before procedure</i>	<i>Culture after procedure</i>
(1) ∞ M. Tetragenus	(1) ∞ M. Tetragenus
(2) Many Gram-negative cocci	(2) Many Gram-negative cocci
(3) A few green streptococci	(3) A few green streptococci
(4) A few grey streptococci	(4) A few grey streptococci

Comment: No demonstrable qualitative or quantitative change.

SUMMARY

In summary then it appears that it was found impossible to alter the character of the flora of the tongue as revealed by aerobic surface cultures by any of the above procedures. Many organisms were undoubtedly removed, but the experiments show clearly that the normal mucous membranes of the tongue can not be washed free of bacteria. It seems fair to conclude, therefore, that the organisms which are found in the normal mouth so uniformly as to constitute in a sense a normal flora are not growing free in the secretions but are in actual and vital contact with the mucous membrane.

A study of stained sections of the tongue shows a mucous membrane not unlike the skin in structure. The covering layer of stratified squamous epithelium is thrown up into microscopic folds and the actual living surface is more or less covered by desquamated shrunken cells which again suggest a cutaneous keratosis. Furthermore, the mucous membrane is everywhere perforated by the orifices of the minute mucous glands. We believe that the actual niduses of growth of the bacterial flora is in these crevices in the epithelium and in the gland orifices. Here there is a localization of the bacteria. As they multiply, certain organisms are thrown off and are removed just as are foreign particles or bacteria experimentally introduced. Hence we have a constant stream passing out from a source which is relatively fixed and therefore allows the persistence of a bacterial species on the normal mucous membranes of the mouth for indefinite periods of time.

If the above conclusions are correct, it seemed probable that a foreign organism placed on the tongue could be readily washed away, inasmuch as there was no actual growth in the superficial layers of the mucous membranes. The next experiments were aimed at settling this point.

EXP. X. The entire twenty-four growth from an agar slant of *Sarcina lutea* was smeared on the anterior part of the tongue. A culture was immediately made. The tongue was then vigorously scrubbed with water for five minutes and another culture was made.

<i>Culture immediately after application of Sarcina</i>	<i>Culture after washing for five minutes</i>
(1) Innumerable colonies of <i>Sarcina</i> —plate covered with a confluent yellow growth.	(1) 2000 colonies <i>Sarcina lutea</i> . (2) The usual tongue flora in normal proportions.

Comment: In contrast to the preceding experiments it was possible in five minutes to wash away the vast majority of the artificially implanted organisms.

Exp. XI. A similar experiment was performed, a twenty-four hour agar slant of *Staphylococcus albus* being employed.

Control culture	Culture immediately after applying albus
Usual tongue flora (no Albus)	Innumerable colonies of albus—confluent growth
After washing for 5 minutes About 200 colonies albus + usual mouth flora	After washing for 10 minutes About 100 colonies albus + usual mouth flora

Comment: Washing for five minutes removed almost all of the *Staphylococci* introduced, although even after ten minutes a few colonies still were present.

DISCUSSION OF EXPERIMENTS X AND XI.

It is seen that even ten minutes vigorous washing did not serve to remove all the colonies of a foreign organism experimentally applied. The reduction being so vast and striking, however, in contrast to the response of the indigenous organisms under similar treatment, it may fairly be concluded that an essential difference exists in the relation to the mucous membrane of an organism adapted to growth in the mouth and a foreign organism experimentally introduced. The latter behaves like an inanimate foreign particle.

The next point to be tested was the following. If the actual growing and multiplying fraction of a mouth inhabitant such as *Streptococcus viridans* is located in the mucous membrane and if there is a balance of power at this point among the various members of the flora which regulates their relative proportions, the reintroduction of an autogenous strain in large quantity should be followed by a rapid disposal of the organisms introduced just as is the case with foreign bacteria. This question was approached as follows.

A culture was made from the tongue of Individual B. The predominating strains of green streptococcus and of *M. tetragenus* were isolated.

Exp. XII. A culture was made from the tongue and immediately thereafter the twenty-four hour growth from a blood-agar plate of the autogenous strain of *S. viridans* was placed on the tongue. Cultures were made immediately after application and two hours later.

Control culture before inoculation	Culture immediately after inoculation with <i>S. viridans</i>
Usual tongue flora	Plate almost pure <i>S. viridans</i>
Culture two hours later	
Same organisms as in control and in about same proportions.	

Comment: The autogenous strain of *Streptococcus*, when re-introduced, did not colonize, but was eliminated just as were foreign organisms.

Exp. XIII. Procedure similar to that of Exp. XII, an autogenous strain of *M. tetragenus* being employed.

Control	Culture immediately after inoculation with <i>M. tetragenus</i>
Usual tongue flora	Plate almost pure <i>Tetragenus</i>
Control two hours later	
Same organisms as in control and in about same proportions	

Comment: Result similar to that in Experiment XII.

DISCUSSION OF EXPERIMENTS XII AND XIII.

In previous studies in which foreign organisms (*B. coli*, *Staph. albus*, *B. influenza*, *B. Friedländer*) were experimentally applied to the tongue, it was found that such organisms were rapidly eliminated, usually within a few hours.² From the present experiments it appears that a culture of an autogenous strain, when reintroduced, is eliminated just as is a foreign organism. It may be that even a single subculture on artificial media alters the bacteria in such a way that its adaptation is lost, but it seems more likely that as soon as the organism is withdrawn from its actual nidus of growth it is no longer able to reattach itself but is doomed to elimination. Be this as it may, we wish to use this experiment merely to emphasize further the point that the persistence of any organism on the buccal mucous membranes implies a close anatomico-biological reaction at the site of growth.

The facts brought out so far deal mainly with the so-called "normal" flora of the mouth—namely, groups of organisms so constantly and persistently present in healthy people that one must assume a high degree of general adaptation between the race of organisms as a whole and the population at large.

There exist, however, other groups of bacteria which are present much less frequently, and yet are often encountered in the normal mouth. Pneumococci, for example, are found in from thirty to fifty per cent of the general population, influenza bacilli in from twenty to thirty per cent, and hemolytic influenza bacilli in from thirty to fifty per cent. Now, although these organisms as a whole are clearly less highly adapted to growth on human mucous membranes than those considered above, the question remains open as to whether in the individual harboring them a different type of adaptation exists. More specifically stated, can the influenza bacillus or the pneumococcus, when growing in the mouth of a healthy person, be more easily removed than the members of the "normal" flora?

This point was a rather difficult one for systematic experimental approach, but certain suggestive observations were obtained. It was found that individual B was carrying hemolytic influenza bacilli on the tongue. Various attempts were made to remove this organism as follows:

- December 8—Tongue scrubbed with water five minutes.
- December 12—Tongue scrubbed with saturated salt solution five minutes.

December 13—Tongue scrubbed with saturated salt solution fifteen minutes.

December 16—Tongue scrubbed with saturated sodium bicarbonate for fifteen minutes.

Cultures made before and after the above procedures showed hemolytic influenza bacilli in similar numbers. On January 3, however, these organisms disappeared spontaneously, nor were they recovered thereafter.

This observation is in accord with the assumption which seems inevitable to us that any organism which persists on a normal mucous membrane for more than a few hours is in vital biological relationship to the mucous surface. Were this not so, it would be removed as a foreign particle by the normal eliminative mechanism. The bearing of this principle on the significance of the "carrier" in the spread of respiratory disease will be discussed at another time.

SUMMARY

The above experiments clarify some of the puzzles of mouth bacteriology. They explain, in the first place, why the organisms which have been regarded as members of the "normal" flora are able to persist and are not eliminated as are foreign bacteria experimentally introduced. They persist because their nidus of growth is actually in the mucous membrane—the organisms which are discharged from these niduses are already doomed to be removed. The situation is crudely analogous to that of the blood cells which are swept away from the parent cell-nests in the bone marrow. Indirectly, we also get the explanation for the fact, which at first seemed remarkable, that foreign organisms experimentally introduced into the mouth are so completely and rapidly eliminated, for it becomes apparent that to persist such organisms need more than a favorable salivary medium—an actual adaptive reaction with the mucous membrane, which is doubtless a biological process of the highest complexity and subtlety, must take place. The question of the importance of the saliva in the growth of organisms in the mouth is also clarified. The saliva, as a rule, plays no significant part either favorable or inhibitory. It now becomes intelligible why bacteria, such as colon bacilli, which thrive in saliva *in vitro*, are not found in the

normal mouth, whereas organisms such as pneumococci or influenza bacilli, which die in saliva *in vitro*, are frequently present. The colon bacillus, despite its growth in saliva, is unable to react with the mucous membrane so that colonization there can take place; pneumococci, on the other hand, are able to do so, and their actual growth processes probably occur in the mucous membranes largely or entirely independently of overlying secretions. It is readily apparent why an organism located in a focus of infection does not spread from such a focus over the entire mouth and throat, and it is clear why an excess of an autogenous strain when reintroduced is promptly eliminated. No sooner has the organism been discharged from its nidus of growth than it is already altered to such a degree that return to colonization is unlikely. It becomes clear, then, that these biological adaptations are most delicate and sensitive, and further analysis may throw light on the essential mechanism of infection and of the production of the carrier state.

Furthermore, it becomes apparent that to speak of a "normal" flora is only appropriate in a broad clinical sense, indicating groups of organisms widely and constantly disseminated and usually harmless. From the biological point of view any organism which has established actual growth on a normal mucous membrane is, for the time being at least, just as normal an inhabitant as any other bacterium similarly adapted.

CONCLUSIONS

1. No bacteria of any sort entering the mouth can persist free in the secretions for more than a few hours.
2. More permanent presence implies a biological adjustment to growth on the mucous membrane whereby the organisms become localized at the site of growth.
3. Such adaptive power is relatively constant for large groups of bacteria in relation to the general population, but varies widely in the case of individual organism and individual host.

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THE CHANGES IN THE OMENTUM OF THE RABBIT DURING MILD IRRITATIONS; WITH ESPECIAL REFERENCE TO THE SPECIFICITY OF THE MESOTHELIUM

By R. S. CUNNINGHAM

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The ease with which cells can be observed in the omentum, without the necessity of subjecting them to much manipulation, has caused this structure to be widely utilized in studying special characteristics of individual types of cells and their interrelationships, under both normal and experimental conditions. In a general way the histological investigations which have been conducted on the omentum can be divided into two groups. The investigations in one of these groups have been concerned with the genetic classification of the cells which constitute the *tâches laiteuses*, particularly with regard to their hæmatological relationships. The other group includes numerous studies which have been made on the characteristics and relationships obtaining between the three great groups of cells which constitute the general framework of the organ—the clasmatocytes, the fibroblasts, and the serosal lining cells. The serosal lining cells have received particular attention in this regard and much work has been done on their reaction to irritants and on the rôle played by them during the formation of adhesions.

The study of the lining cells covering the omentum has three salient features: the relation of these cells to the production of the free cells which are found in the exudate of the peritoneal cavity during inflammations, the relation of the serosal cells to the clasmatocytes, and finally, the relation of these lining cells to the fibroblasts which constitute the greater part of the framework of the organ. The first two questions are closely related and have been discussed elsewhere;⁹ and the conclusion was reached that the majority of the free cells of the exudate were derived from the clasmatocytes, which, in the omentum, occur chiefly in the neighborhood of the blood-vessels and in the *tâches laiteuses*, though they are also to be found scattered through the entire omental network. The serosal lining cells were found to have no part in the formation of the true phagocytic mononuclears of the exudate, and no genetic relationship with the clasmatocytes.

In regard to the relationship of the lining cell to the fibroblast there has been much diversity of opinion; many of those who have studied the omentum during inflammatory reactions have concluded that these cells are capable of definite interchange in morphological and functional characteristics. Others have described similar

appearances in the omentum but have left unsettled the question of the interchangeability of fibroblasts and serosal cells. And finally, a few have maintained the specificity of the serosal cells and the fibroblasts in the general serous membranes, but without any direct consideration of the omentum itself.

Ranvier^{20, 21} was the first author to discuss the relation of the lining cells of the omentum to the other cells constituting the framework of that organ. His views have been widely accepted, and his conclusions supported by the observations of many workers; Cornil,⁸ Marchand,^{18, 19} Roloff^{24, 25} Borst,¹ and others. Ranvier describes the omental lining cells as changing during a mild inflammation from characteristically outlined pavement cells to star-formed cells with long processes which he considers as wholly indistinguishable from the fibroblasts, and he concludes from such observations that these two types of cells are identical, at least so far as their reactions to irritation are concerned, and hence must be capable of easy interchange.

Among the more modern workers on this subject are Weidenreich³⁰ and Schott;²⁶ these observers, after long and minute study of the reactions of the peritoneal membranes, conclude that the lining cells of the omentum cannot be distinguished from the fibroblasts and that the two types of cells are entirely interchangeable. Schott, in his study on the cells of the serous cavities, derives the free cells of the peritoneal exudate from the serosal lining cells and the fibroblasts indiscriminately.

Kiyono,¹⁵ on the other hand, after study of omental spreads and sections from animals vitally stained with carmine and trypan blue, states that he could never find any indication of a transition from lining cells to fibroblasts. He says that normally the lining cells form a continuous, characteristic cell-series over the entire omental surfaces, and that they can easily be differentiated from the fibroblasts. But he states that during inflammation a portion of the lining cells assume a form which greatly resembles that of the fibroblasts, and he therefore admits the possibility of a close relationship between the two. He is unable to state with certainty if the lining cells do, or do not, participate in the formation of new connective tissue.

Marchand's¹⁸ description of the omentum in fresh preparations is very exact and deserves considerable

attention and credit. He studies the omentum after the introduction of a suspension of lycopodium spores, and describes the mesothelial cells, seven hours after the injection, as rounding up and their cytoplasm becoming finely vacuolated; these cells stand out clearly from the surface of the omental network. Twenty-four hours after the injection he found many delicate protoplasmic structures of various forms: flat, irregular masses of protoplasm with numerous processes which appeared and disappeared and long spindle-formed cells with finely vacuolated protoplasm. He states that the origin and relationship of these large, variously formed elements could not be determined in fresh preparations. From stained sections and preparations from later stages he concludes that all of the lining cells proliferate to form fibrillary structures, and later a part of these form fibrils and new connective tissue, the other part forming more lining cells. The former are the star-formed irregular cells, to which he assigns amœboid capabilities and which he believes, participate with the clasmatocytes in the formation of giant cells, while the latter become again the usual flat mesothelial elements.

Büttner⁵ introduced cultures of staphylococcus aureus or weak silver solutions into the peritoneal cavity of guinea-pigs to produce inflammations. He studied the omentum carefully at varying intervals following this procedure and says, because of the complicated structure of the omentum, it is difficult, after irritation, to differentiate between the types of cells or decide which groups are undergoing mitosis. Although he leaves open the question of a possible transformation of lining cells into fibroblasts, he thinks his experiments show that the omentum is covered by a single layer of cells which react during inflammations as true epithelium. This was particularly well demonstrated in his sections cut perpendicularly to the surface where the differences between the cells on the surface and those constituting the sub-serosal tissue are very striking. In sections taken three days after the commencement of the inflammation he found areas showing evidences of regeneration of the lining cells. After seven days he found adhesions where the lining cells had entirely disappeared. Where they had regenerated there were no adhesions.

Dominici^{11, 12} thinks the cellular part of the omentum is composed of several syncytiums; the syncytium of lining cells, of fibroblasts, of adventitial cells, of fat cells, etc. These syncytiums are not entirely separated from one another, but are connected by means of anastomoses between the cells. Scattered through the whole structure are free cells among which he classes the macrophages. These macrophages arise by differentiation from the various syncytiums which belong collectively to the connective-tissue series. According to him, the endothelial syncytium of lining cells is to be considered as modified

connective tissue, which during inflammation gives rise to a large number of macrophages.

Von Büngner⁴ thinks that both serosal lining cells and fibroblasts participate in the formation of granulation tissue, but he found that they retained their individual characteristics during the process, and that the cells derived from them could be easily differentiated into young connective-tissue elements and young lining cells. He could not detect any transition of lining cells into fibroblasts, but he thinks that the lining cells play a more important rôle in new tissue formation than is usually attributed to them.

Von Brunn,^{2, 8} after minute study of inflammatory processes in the peritoneal cavity, concludes that the lining cells are of a specific epithelial type which never gives rise to fibroblasts and that young lining cells are never formed from fibroblasts during the processes of inflammation and the formation of new tissue. In referring to the work of the French authors he suggests that perhaps their views were so unanimously in support of a genetic relationship between lining cells and fibroblasts, because they based their conclusions principally upon observations made on spread preparations of the omentum, in which material, Von Brunn states, it is extremely difficult, if not impossible, to obtain a clear picture, the underlying fibroblasts becoming easily confused with the superimposed lining cells.

Tschaschin,^{28, 29} after a prolonged study of the serous cavities with vital dyes, concludes that the serosal cells, because they remain totally unstained, are specific; but in the omentum this specificity is extremely difficult to establish. In connection with this question it is interesting to note that he quotes Maximow as believing that the fibroblasts in the omentum and mesentery retain a more embryonic character than in the general connective tissues, and that it is more difficult to differentiate the fibroblasts from the resting wandering cells in the omentum than elsewhere.

Finally the work of Foot¹⁴ on wound healing in the omentum must be referred to in this connection as it has a certain bearing on the results of these observations. In his experiments he found a very remarkable reversal of type in the case of the connective-tissue cells and of the endothelium to an undifferentiated mesenchyme, from which there arose new vessels, connective tissue, and "phagocytic endotheliocytes." These conclusions are of interest in regard to the relation of serosal lining cells to fibroblasts, because, if these two groups of cells are very closely related, it would seem most likely that the reactions described by Foot would involve the serosal lining cells as well as the connective-tissue cells.

In general, then, it seems that the majority of those investigators who have studied the omentum with particular regard to the question of the changes which take place during irritations have concluded that the fibro-

blastic and mesothelial elements are morphologically and functionally interchangeable. But this view has not been accepted by others, and the principal evidence in favor of the specificity of these cells has been obtained from the study of sections of the serosal membranes covering the various organs. The contradictory conclusions arrived at may have been the result of the various methods employed, or due to some fundamental differences between the cell potentialities as they appear in the omentum and in the general subserosal tissue, as suggested by Maximow.

It has been long known that the serosal lining cells underwent changes in size, shape, and general appearance when subjected to irritations. In attempting to determine the exact nature of these reactions it has seemed essential to reinvestigate both the omentum and the other serosal membranes with every available method. The reactions of the mesothelial cells are obviously important in two connections; the fundamental relation which they bear to the fibroblast and in regard to the rôle which they play in the formation of adhesions. The present communication reports the results of the examination of the omentum in an effort to determine whether the changes which occur in the morphological and cytological characteristics of the serosal lining cells, during mild irritations, indicate any genetic relationship, or functional similarity, between these cells and the fibroblasts.

The structure of the omentum of the rabbit is very characteristic. There is a framework of blood-vessels which anastomose freely without the intervention of a very elaborate capillary bed, that is, much of the circulation is arterio-venous in character. The larger vessels are surrounded by layers of adipose tissue and, in this way, the omentum presents the appearance of a flat membrane marked off by vessels and fat into irregular sections, or areas, of quite diverse sizes. Rich²³ has shown, however, that there is a capillary bed which is not apparent with the usual methods of observation, but which becomes very prominent after the administration of histamine, which by dilating the capillaries makes them far more conspicuous. But not even after the administration of histamine can a very elaborate plexus of capillaries be observed in the thin membranous areas ordinarily outlined by the larger and medium-sized vessels. That these areas are non-vascular is far from true, but the vascularity is sufficiently decreased at these places to make study of the membrane as a whole entirely satisfactory.

The thin membranous part of the omentum consists of numerous cells surrounded by a mass of interlacing connective tissue fibrils and covered by a layer of flattened mesothelial cells, whose outlines, in the normal state, can always be demonstrated by the mosaic pattern which they evidence after treatment with silver nitrate and sub-

sequent exposure to sunlight. The special structures which are so evident in the omentum of the rabbit, called the *tâches laiteuses*, are scattered more or less indiscriminately throughout the entire omentum. The *tâches laiteuses* are collections of various types of free cells grouped about areas of increased vascularity. These cells have been widely studied in connection with the formation of free cells during inflammatory reactions in the peritoneal cavity, and in relation to general hæmatological problems.

The omenta upon which the observations reported here were made were from a series of rabbits which had had their peritoneal lining cells irritated by the injection of heterogeneous laked blood. Some of these rabbits were vitally stained by the intravenous administration of trypan blue, and a few received the vital dye intraperitoneally in order to give the irritated serosal lining cells every possible opportunity to absorb and store the dye.

When the animal was ready for study it was anesthetized and the abdomen opened; the omentum was observed with regard to the general appearance, occurrence of adhesions etc. Several small pieces were transferred to coverslips, which were then immediately sealed to the slide with vaseline; these preparations were sometimes stained with neutral red by Pappenheim's method. In many cases small amounts of fluid were found in the peritoneal cavities of the rabbits and in such cases a small drop of this fluid was used to mount the fragment of omentum; in others Locke-Lewis solution or homologous serum was used.

The remainder of the membranous part was carefully spread without touching the surface, a coverslip was dropped on it and the area cut out; the coverslip with its layer of omentum was then immersed in a weak solution of silver nitrate for a few minutes, transferred to distilled water and exposed to direct sunlight until it showed a light brown; it was then fixed in 95% alcohol. Other preparations were rinsed carefully before being placed in silver nitrate, and still others were fixed immediately in alcohol, sublimate, or osmic vapor. From each animal at least three preparations were always made, one to be studied fresh, one silvered, and one fixed directly for control.

In the normal omentum, when the fresh preparation, the ordinary stained spread, and the spread treated with silver nitrate were compared, the remarkable fact was noted that in the two former the mesothelial layer of cells could not be made out at all. There were nuclei which were suggestive, but these were in no case sufficiently distinctive to be diagnostic. As soon, however, as silver nitrate was applied, the superficial layer became entirely apparent and distinctly separated from the other cells which were subjacent to it. It is entirely obvious that if in the normal, fresh omentum the cells could not be distinguished with ease, those in the irritated omen-

tum would be still more difficult to classify, so that the application of the silver method becomes even more important after irritation. In all studies on the fresh omentum it was impossible to determine the outline of the serosal lining cells, and hence the various types of cells present were very difficult to distinguish. In fresh unstained omenta from animals which had received two or three doses of laked blood the microscopic appearance was quite bewildering. In such a preparation only the nuclei and large numbers of granules surrounding them could be seen clearly. The cellular outline could be made out only in rare instances, and analysis of the type of cell depended entirely upon the appearance and reactions of their granular content. But by means of various vital and supravital dyes and by numerous control methods applied to the fixed material, the characteristics seen in the fresh could be analyzed to a considerable extent.

Three types of cells could always be observed. The first two were quite similar in many respects and were sharply differentiated from the third by certain characteristics of the vacuoles and granules which they contained. The two that resembled each other so closely had in common large, pale nuclei with one or two very highly refractive nucleoli, and contained varying numbers of highly refractive granules, all of which were of the same size and character. The arrangement and number of granules, however, within each cell permitted a division of these cells into two groups; one group having many granules which were usually distributed in one solid mass of quite varied shape, sometimes a crescent about one side of the nucleus, sometimes a belt surrounding the nucleus, and in a few cases they were arranged in several clumps about the nucleus. In all of the cells of this type the highly refractive granules were associated closely with the nucleus and were never seen branching out through the cell in any process-like arrangement. In the other type, this finding was just the reverse; the granules were scattered in quite irregular fashion from the nucleus, sometimes in a single, long, slender thread extending from each end of the nucleus, and again, in several irregular processes of uneven size and length, suggesting the spread legs of some arachnida. In this last type the granules were fewer in number and often formed very slender and detached threads extending throughout the finer processes of these cells. These two types of cells were, respectively, the serosal lining cells and the fibroblasts.

The third type of cell was the clasmatocyte, a cell whose character was indicated quite definitely by the great variation in the size of the vacuoles and granules which it contained. There were only a very few of the fine, highly refractive granules, but there were vacuoles of various sizes, and masses of hyaline material which were evidently phagocytic enclosures. The arrangement of these cellular enclosures was quite irregular, but

seldom extended as in the fibroblast into fine lines or threads; they were rather blunt and rounded as though in true pseudopodia. Most of these cells were rounded, however, and appeared as balls or spheres of massed granules and vacuoles surrounding the nucleus.

When a preparation such as that described above was mounted on a coverslip previously prepared by being coated with neutral red, the three types of cells presented a very different appearance. The clasmatocytes took up large amounts of the dye into their vacuoles and became masses of red globules of different sizes with a few interspersed, fine clear granules; while the other two types had only a few, fine, red droplets scattered among the clear granules. In the fibroblasts there were more red bodies than in the serosal cells and they were especially prominent in small enlargements of the fine processes.

The application of silver nitrate to the study of the characteristics of the omental cells during inflammatory changes proved to be extremely difficult to carry out successfully, because it was found that a certain amount of fibrinous and cellular debris always accumulated on the surface of an irritated area and caused irregular staining with the silver. The use of silver nitrate to outline the margins of endothelial cells was first described by Von Recklinghausen²² and has since proved a very important part of the technique in the study of blood-vessels and the linings of the serous cavities. What the nature of the reaction is, is yet in doubt. It was first thought to represent the impregnation of some intercellular cement substance which had the power of combining with the silver. It has since been suggested that the reaction was more likely due to a peripheral concentration of chlorides in the cells and that the silver oxychloride which is formed is reduced in sunlight so that metallic silver is precipitated in the borders of the cells. This entire question is thoroughly reviewed by Macalum.¹⁷

The cellular debris which is formed during the course of an inflammation increases as the intensity of the irritation is augmented, and in this way the application of the silver reaction to the irritated omentum becomes increasingly difficult. It must be noted here that the successful application of this method could not have been considered as plausible until the theory of peripheral salt concentration had been suggested. I have found that if the technique be carried out very carefully the method may be used to great advantage in the study of mildly irritated omenta. When the silver nitrate was applied in very weak solutions, $\frac{1}{8}$ to $\frac{1}{4}$ %, and the spread preparations were agitated gently while in the silver solution, and washed carefully before being exposed to the light, in some of the specimens the entire surface of each serosal cell had a brown appearance with a darker outline, and the entire layer of rounded or irregular cells became apparent. The use of this reaction to demonstrate

the stages in the changing mesothelial cells, during the progress of an irritation, has proved quite successful and has established certain definite characteristics as most specific.

The first change observed in the layer of serosal cells was an irregular widening of the line which marked their boundaries. This took place very irregularly so that there was no longer a perfect apposition of the cells each to each. In an omentum which had been subjected to a mild irritation there were many places where one could see groups of surface cells with perfect apposition, side by side with other groups in which considerable gaps occurred between the cell margins. This is shown in Figure 1; on the left of the figure there are several mesothelial cells which have outlines, changed only very slightly from the normal; while on the right the cells are beginning to separate and there are numerous spaces between them. This type of reaction is entirely natural in a structure like the omentum, because consistent changes could never be expected over the entire surface at one time. Every stage of separation of the cells from small gaps in the thin, continuous, brown line of silver precipitate, seen in the normal omentum, to areas the width of nuclei were observed, but in these early stages of irritation there were almost always points of contact except where an occasional cell had been desquamated. In many places the cells were bound together by numerous, fine processes as seen between the two cells shown in Figure 3.*

At the same time that the withdrawal of the borders of the cytoplasm of the serosal cells was taking place there was a beginning increase in the thickness and density of the cytoplasm. This rounding up of the serosal cells has been often observed in the serous membranes in general, but in the omentum the changing mesothelial cell has usually been confused with the branching fibroblast. The cells shown in Figure 2 have separated in part from each other and have increased somewhat in thickness

* This brings up the question of whether the surface cells form a true syncytium, as claimed by Kolossow, or whether these cells are merely in apposition, as is the case with many tissues. Kolossow¹⁶ describes the lower part of the serosal cells as anastomosing with the neighboring cells by means of fine processes, while the upper part of the cell merely lies in apposition to the surrounding cells. If this opinion prove correct, it will be easy to understand the drawing apart of cells with the formation of multiple, small openings between the anastomosing processes. It is evident that the observations cited above, at first sight, strongly support Kolossow's idea that the surface cells are bound together by numerous processes. But long and careful study of large numbers of preparations has not convinced me that these are anastomoses in the sense of syncytia, but rather served to indicate a union of a less intimate character. There is, however, very little doubt but that there is some type of junction which binds these cells together more closely in some places than in others. The nature of this union has so far not been analyzed, and is a most difficult question to settle.

but have not changed very materially from their normal, flat morphology. Here and there in early stages a single cell was found to have been desquamated and a larger area than could have been formed by mere separation was discovered, but other places were to be seen in every preparation where the cells were regularly spaced as if they had each retained their normal positions and merely had become more and more rounded up. In these early stages of irritation the processes which the serosal lining cells manifested were very striking and were quite characteristic, varying from cell to cell only in the matter of size. They were seldom very long, though they occasionally reached 8 to 10 microns in length. They were sharply pointed, somewhat similar in shape to the old-style church steeple. They generally extended at right angles to a line drawn at their base tangential to the periphery of the cell, and in cases of more rectangular cells were perpendicular to the sides. These projections were usually fine, very sharply-pointed, and about 3 to 5 microns long, although, as already stated, they were sometimes even longer. Very occasionally the ends were blunt or forked instead of pointed, but this was not very common, and neither did these processes have bulbous enlargements on them, nor were they divided to form two, definite, characteristic branches. These processes are well illustrated in Figures 2, 3 and 4. Figure 3 is a microphotograph of a preparation in which a large rectangular cell was becoming separated from a smaller one, and the processes which still hold them together are well shown. In Figure 2 there are to be seen the fine, long processes characteristic of cells which have but recently separated from each other. It is important to note here that almost every individual preparation of an omentum from an animal with relatively mild peritoneal irritation showed several stages of the progressive changes which represented the course of an irritation. This is easily understood because the omentum may be exposed in part, and protected in part at the same time. In every specimen, however, there was a general preponderance of a certain type, and in this way it was easy to establish the characteristics which represented the several different stages in the process. I mention this because it is so very easy to misinterpret histological appearances and it is essential to be assured that the particular area which is being examined represents a definite stage in the series of events which are being studied.

In the stage following the one described above the mesothelial cells had continued their increase in thickness and the rounding up of their cytoplasm, and therefore the edges of the cells were more widely separated. As this rounding up progressed, the number of processes on the cells diminished in number, but those that remained were in general somewhat larger. One cell in Figure 4 has a process of this type. In this stage of the irritation the omentum was often covered in large areas

by cells scattered very regularly, but separated from each other by quite considerable areas of denuded tissue. Figure 4 is from such an area and here it is quite certain that no cells have been desquamated. In Figure 3 it is probable that cells have been desquamated, as the distances between the cells appear greater than usual for so early a stage. In preparations from animals which had been exposed to still longer irritations the mesothelial cells had rounded up still further, they had been desquamated in a greater degree, and were in large measure obscured by the numerous clasmatoocytes which had reached the surface and were adhering to it, as well as by some cellular debris. The processes of the serosal cells had not materially changed in type, but were somewhat more irregular than in earlier stages, probably due to the attempt of some cells to flatten out and recover the denuded areas while others were continuing to round up as the result of the continued irritation. Mitotic figures were extremely difficult to find in preparations of spread omenta, and the evidence of cellular division was based upon sections taken from the same omenta and appropriately stained. Active mitotic figures were relatively few even in these sections but many cells were to be seen in various stages of division.

When the silver preparations are successful, they are very brilliant and demonstrate that the morphological changes which take place in the mesothelial cells are wholly characteristic and indicate the specificity of this cell-type. When the technique outlined above was combined with previous vital staining the definition of the serosal lining cell as a specific type became even more exact, since the method of storing the vital dye was equally as specific as the morphological changes.

In all of the omenta from animals vitally stained with trypan blue, the mesothelial cells showed very interesting reactions. In the normal these cells have been found to store the dye in a rather characteristic manner, the dye being collected in a small clump in a definite part of the cell; on progressively increased staining the dye granules extended around the nucleus in a perinuclear rosette. The normal staining of the serosal lining cells has been described in detail elsewhere.¹⁰ Studying the reaction of the cells which had been irritated, the deposits of blue were found to deviate somewhat from those in the normal. As the cells began to separate and round up there was a tendency of the blue droplets, which had been rather specifically located in one end of the cell, to be dispersed somewhat more widely in the same general part of the cell. In Figure 2 the blue granules can be seen as groups of definite black spots in one end of each cell, *i.e.* opposite one pole of the oval nucleus. But the granules are somewhat irregular in distribution, as may be noted especially well in the two cells in the lower right corner of the photograph. The silver precipitate in a large measure obscures the blue granules in the bodies

of the cells. With increased irritation there was a more general tendency to even distribution in those parts of the cell where there was much blue and a gradual though uneven widening of the belt of blue constituting the perinuclear rosette. In general the effect of irritation was found to be a more even distribution over a larger area of cytoplasm, sometimes but not always, accompanied by a diminution in the concentration in that part of the cell which originally contained a small ring or mass of granules. In Figure 5, which is from an omental spread that had been treated with osmic acid and had not been silvered, there is, in the upper center, just above the round, heavily stained clasmatoocyte, a mesothelial cell which is somewhat out of focus, but which nevertheless illustrates the distribution of the vital dye granules as irritation progresses. The cell is irregularly triangular in shape, the upper part on the left being entirely out of focus, while in the upper right there is a very distinct ring of blue granules. This ring represents the first dye deposited in the cell while the patches of granules in the remainder of the cell have been formed during the progress of the irritation.

It is extremely important to note that in no case were there any granules of blue to be seen in the processes of the serosal cells. In fact, in those cells having the largest processes there was not the least tendency of the blue granules to enter them, and in this particular there was a very sharp contrast to the fibroblasts. In Figure 4 there are two cells which have very characteristic processes, and these show very clearly that they contain no dye inclusions. These clear processes should be contrasted with the long, branching, dye-filled processes of the fibroblasts in Figures 5 and 6.

The reactions of the fibroblasts and the clasmatoocytes in these omenta from animals having mild irritations were wholly characteristic. Scattered throughout the omentum characteristic fibroblasts were to be found everywhere. The typical fibroblast has been studied and described so often that it seems useless to enter into the especial characteristics of this cell at any length. Schott,²⁶ Büttner,³ Kiyono¹⁵ and Tschaschin^{28, 29} have figured the fibroblasts in the omentum as large cells with smaller nuclei than the serosal cells, fading outlines, and fibrillary cytoplasm. Evans and Scott¹³ have studied them in subcutaneous preparations with numerous vital dyes and figure their vital dye content quite extensively; it is probable that these would be similar in the omental fibroblasts, even though Maximow has suggested that they retain their embryonic characteristics in this locality during adult life. In preparations from a normal omentum which has been vitally stained with trypan blue and then silvered, the fibroblasts were seen in between the two layers of serosal cells as faintly outlined cells with fine blue granules scattered through the cyto-

plasm. These cells were often branched and even though the outlines were difficult to identify exactly, they could be made out to some extent because the trypan blue was distributed throughout the processes. When subjected to the effects of irritation, the fibroblasts instead of becoming more compact developed much more extensive processes which in many cases were quite remarkable. They reminded one very much of the branched cells of the tadpole's tail as figured by Clark and Clark⁷ and in a quite similar manner contained the trypan blue in those enlargements which are found on their processes. In my preparations these cells were especially characterized by the fact that the vital dye extended to the tips of their branches (Fig. 5), although the amount of dye in the fibroblast was very much less than that found in the clasmatocyte. As has been stated, the fibroblasts lie scattered throughout a meshwork of fibrils between the layers of serosal cells. During early stages of any irritation these two groups of cells undergo specific changes without much departure from their definite layer-like arrangement. The mesothelial cells of the entire surface layer have, in many omenta, separated and rounded up, so that each cell was divided from its neighbor by a considerable area; and at a different level there were large numbers of variously shaped, richly branching fibroblasts. This layer arrangement is well illustrated in Figures 4 and 6. Both are photographs from the same omental spread. In Figure 4 there are shown seven mesothelial cells which have separated from each other and rounded up to a considerable degree; if the photograph be studied carefully the shadows of other cells can be seen in the depths. In Figure 6 the camera was focused on the fibroblastic layer and the mesothelial cells may only be seen as faint outlines; one of these can be fairly well made out in the lower left corner, while two others cast somewhat less well defined shadows in the upper right corner. When the irritation was more advanced there was more tendency for arms of the branched fibroblasts to extend into the spaces between the separated mesothelial cells. In Figure 5 the single mesothelial cell is seen to be only slightly out of focus, while there is considerable variation in the relative clearness of the different processes of the two numerously branched fibroblasts. The distribution of the dye in these long, fine, streaming processes was most remarkable, some of them extending entirely across the oil-immersion field, and often they appeared to be attached to or following fibrils of some kind. The contrast between the fibroblasts and the clasmatocytes in Figures 5 and 6 is very striking, particularly in Figure 5; and it must be noted that the characteristics are about equally as specific for the two types of cells in both figures.

In the omentum of a vitally stained rabbit there are, in addition to the fibroblasts, many large cells, most of them long and narrow and loaded with blue granules.

Among these cells are some that are round or oval in shape; these latter are especially numerous in the *tâches laiteuses*. These cells are typical clasmatocytes and have been carefully studied by many observers. Marchand¹⁹ notes their especial affinity for the perivascular regions and calls them "adventitial" cells. Long lines of them are always arranged along the course of blood-vessels and are at times in most intimate contact with the endothelium of the capillaries. This distribution of the clasmatocytes is used by Shipley²⁷ as an argument for concluding that they represent a type of digestive organ, functioning intermediately between the blood-vessels and the tissue-cells proper. In preparations of the normal omentum the clasmatocytes were usually either long and slender cells with centrally located nuclei, or else round or oval cells with somewhat excentric nuclei. The former type is the one invariably associated with the blood-vessels, and so striking is this morphology that they have been termed "trailer" cells by Buxton and Torrey.⁹ In the *tâches laiteuses* the more compact type was often met with, but even here, there were large numbers of the long and slender cells loaded in a striking manner with the vital dye.

When the omentum was irritated, the first change that could be noted in the clasmatocytes was a tendency to round up and change from the long, slender cells to round or oval masses, with or without, short, blunt processes. The surface of any irritated omentum was dotted with these round cells loaded with trypan blue and large numbers could be seen between the serosal layers. Figure 5 shows the typical rounded clasmatocyte, while Figure 6 shows one with blunt processes filled with trypan blue inclosures.

In the study of the omentum during mild irritations by means of the methods outlined above, it has become entirely clear that the serosal lining cells and the fibroblasts react differently both as regards their morphological appearance and the distribution of their vital dye-content. The serosal lining cells increase in thickness and are much more compact than the normal, flat, plate-like structures. They differ considerably in size and shape, being round, oval or square and some of them have a few, fine-pointed processes which vary in size but not in character. The vital dye content is more diffuse than in the normal cells, but there is never any dye to be seen in any of the fine processes. On the other hand, the fibroblast during irritation becomes an elaborately branched structure, forming a marked contrast to the compact serosal cell. The processes of the fibroblasts vary enormously in size and shape; some are long and slender, others branched into two or more subdivisions, and many have bulb-like enlargements, especially where they branch. The vital dye is distributed throughout these processes, the granules often being collected in the bulb-like enlargements.

It is thus obvious that during the course of a mild irritation the changes which take place in the mesothelial and fibroblastic elements indicate that they become even more widely separated from each other in their group-characteristics. The cell-bodies and processes of these two cell-species are morphologically quite distinct in regard to size, shape and general characteristics. It seems entirely justifiable to conclude that such marked differences must denote some variation in the structure of the cytoplasm, and hence indicate some especial adaptation for certain definite physiological activities. In addition to these morphological criteria, it has been demonstrated that the vital dye content of the fibroblast and the mesothelial cell presents a sharp contrast. Here again the principal interest is attached to the wide distribution of dye granules in the processes of the one cell and the total absence of the dye from those of the other. That differences in the way in which vital dyes are stored represent corresponding differences in physiological adaptation seems most probable, and permits further security to be placed in the conclusion that the mesothelial and fibroblastic elements are wholly specific.

It is hoped that the observations reported above, which differentiate the reactions of the mesothelial cell and the fibroblast quite sharply during mild irritations, will be useful in determining the rôles performed by each of these two types of cells in the healing of wounds, the regeneration of denuded areas, the formation of adhesions, and finally aid in establishing the genetic derivation of the so-called "endotheliomata" of the lining membranes of the various serous cavities.

EXPLANATION OF PLATE

All the figures are micro-photographs of omental spreads from rabbits which had had continuous, mild irritations induced by the repeated introduction of laked blood into their peritoneal cavities. The negatives have not been retouched.

Fig. 1.—Exposure to mild irritation for 36 hours. Preparation silvered and stained with carmine. Shows relatively normal mosaic pattern on the left, and beginning separation of the cells on the right. $\times 500$.

Fig. 2.—Exposure to irritation for 60 hours. Preparation from rabbit which had been vitally stained with trypan blue. Silvered and stained with carmine. Shows partial separation and beginning rounding up of the mesothelial cells. The blue droplets are seen as irregular patches in the cells. $\times 700$.

Fig. 3.—Exposure to mild irritation for 4 days. Silvered and stained with carmine. Shows the final stage in the separation of two mesothelial cells and the general characteristics of these cells when definitely modified by the irritation. $\times 1100$.

Fig. 4.—Exposure to mild irritation for 5 days. Vitally stained with trypan blue. Shows the even distribution of the serosal cells on the surface of the omentum; there has probably been no desquamation. $\times 850$.

Fig. 5.—Exposure to mild irritation for 6 days. Vitally stained with trypan blue. Fixed in osmic acid and stained with carmine. Shows two clasmatoocytes heavily loaded with blue granules, two branching fibroblasts, and one mesothelial cell. $\times 700$.

Fig. 6.—Same preparation as Fig. 4. Shows one clasmatoocyte with a large content of trypan blue and pseudopodial-like processes, one characteristically branched fibroblast, and the shadows of three mesothelial cells which are out of focus. $\times 750$.

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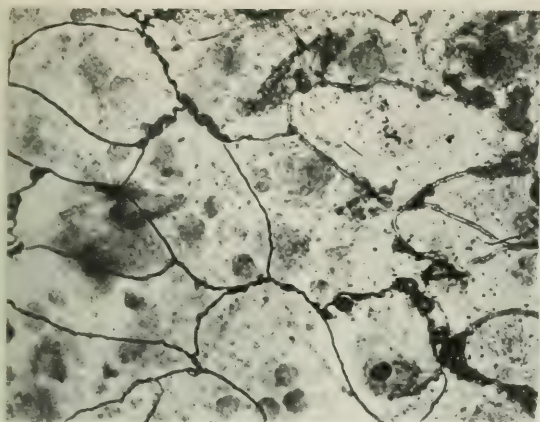


Fig. 1

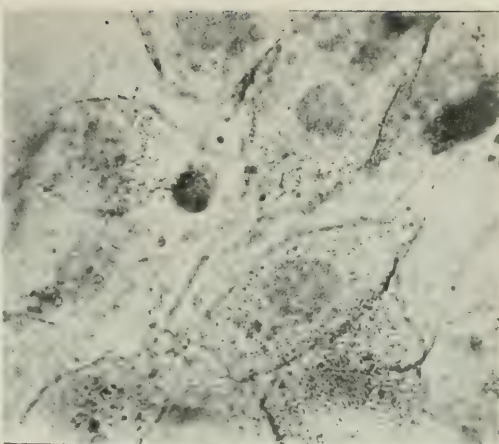


Fig. 2

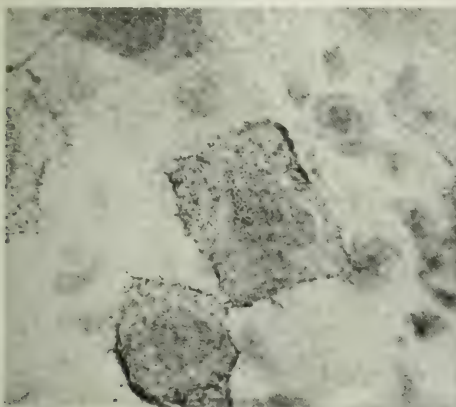


Fig. 3

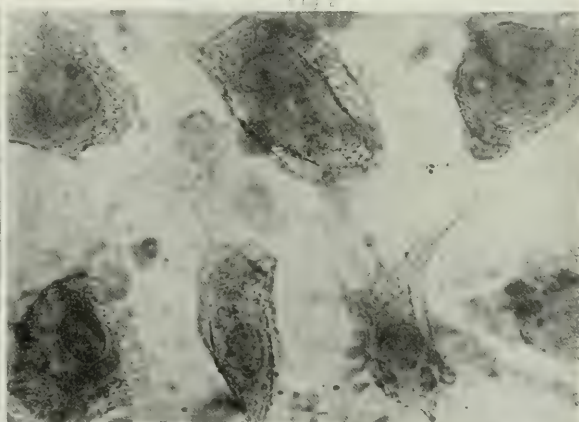


Fig. 4

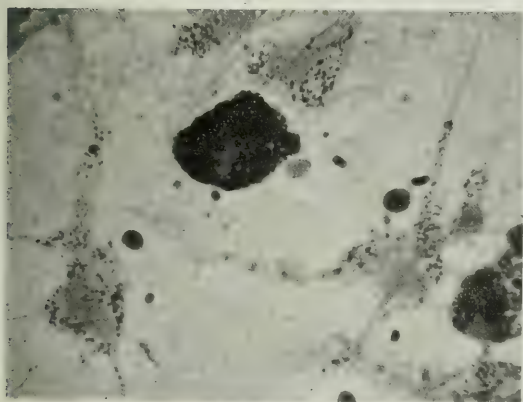


Fig. 5

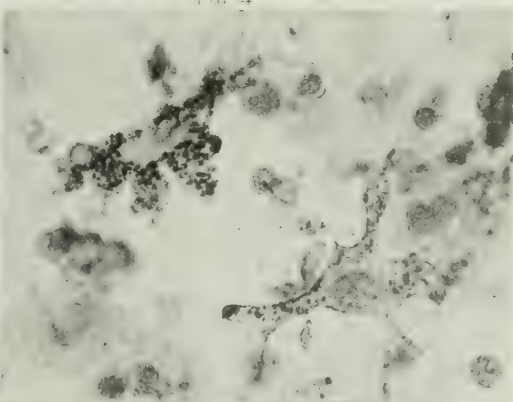


Fig. 6

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THE ELEMENTARY SCHOOL AND THE INDIVIDUAL CHILD^{*}

(ABSTRACT)

By ESTHER LORING RICHARDS

(From The Henry Phipps Psychiatric Clinic of the Johns Hopkins Hospital)

In *Mental Hygiene* for April, 1920, the writer described a fifteen months' study of "Some Adaptive Difficulties Found in School Children." The work was a bit of private research in School No. 76 of the Baltimore schools, and quite divorced from any organized activity in the public school system.

Of the forty-six children who came under observation, thirty-five were reported as having difficulty in keeping up with their grades in one or more subjects. In each of these cases where there was a suggestion of retardation, the Binet-Simon test was applied. As a result, sixteen of the above-mentioned thirty-five were found to have a mental retardation of from three to six years. The academic troubles of the remaining nineteen were associated with, if not the disguised expression of, such faulty psychobiological reactions as shyness, laziness, inattention, and vicious tendencies, sensitiveness to criticism, daydreaming, hypochondriacal fears, with resulting irregular attendance. The eleven remaining from the total forty-six were referred for the more overt adaptive difficulties of temper tantrums, sullenness, crying spells, twitching, indifference, excitability, poor coordination with the hands, quarrelsomeness, etc.

In reviewing the data accumulated, one fact stood out; in practically every case the peculiar characteristics for which the child was referred could be easily traced to their first appearance in the early years of school-kindergarten and first and second grades. In the majority of

cases, also, the unhealthy habits of adaptation began in the home, and were carried into and through the school life, handicapping the efforts of teacher and child to get together on the business of early training in the classroom; and the commonest result of this handicapping was the repetition of grades. Accordingly, it seemed best to devote some time to the first grade, with the special object of examining the problem of backwardness at its source.

By way of experiment, eighteen children were selected from the first grade for special study. Their ages ranged from six to ten, and their years of repeating the first grade from one to three. These children were studied from the standpoint of the story of the home, the story of the school, and the story of the child himself, as recorded under the headings of complaint, school history, habit data, personality traits, and a rough estimate of physical status as obtained from the health records, weight curve, and brief examination. Aside from the Binet-Simon tests, no special technique was used, either in the examination of the children or in the sifting of facts. The Binet-Simon findings were as follows: In two cases the actual age and tested age were exactly the same; in seven cases there was a difference of only one year between the actual age and the tested age; in seven cases there was a difference of two years between the actual age and the tested age; one child objected so violently to examination that it was thought best not to push matters; and one child showed a difference of three years between the actual age and the tested age. It was felt that the difference of from one to two years between the actual age and tested age of fourteen of these

^{*} Read before the Mental Hygiene Section of the National Conference of Social Work, Milwaukee, June 23rd, 1921. Published in *Mental Hygiene*, Vol. 5, No. 4, pp. 707-723, October, 1921.

children represented no real backwardness, but was the expression of various factors in the background, development, early training, and personality traits of the individual children. That these were the facts responsible for the lodging in the first grade seemed more probable than deficient mental equipment.

Turning from the facts of standardization to those of physical condition and living arrangements, there was nothing striking in the story of these little people. There was one mouth breather, one child with definite eye strain, and two tuberculosis suspects. Several children appeared pale and undernourished, but only two weight curves were below the normal. The habit data were characteristic of this industrial section—comparatively late bedtime, with two, often three sleeping in a bed; a diet of coffee, buns, and soup; movies two nights a week. Unideal as these conditions are, it must be remembered that they have not proved a serious hindrance to the school progress of hundreds of other boys and girls.

These eighteen candidates for reconstructive therapy were put under a special teacher in September, 1920. In June, 1921, ten members of this class were qualified for the third grade and seven for the second grade. Only one child of the class failed to respond to special study.

To what have these results been due? Before giving my own impression of the matter, let me say a word about the school background of the above experiment. For several years School 76 has been studying the problem of repetition in its midst. For three years a special health worker, privately financed, devoted her whole time to following up minor ailments—common colds, post-contagious-disease conditions, and any other factor influencing regular attendance. The result was an attendance record of from 96 to 98 per cent, but the repeating of grades remained *in statu quo*. This same worker directed a campaign towards the relation between progress in school and the clearing up of tonsils and adenoids. The results, over a period of three years, were that 50 per cent of the children operated on showed no difference in their school work, 25 per cent showed some improvement, and 25 per cent appeared to make a poorer showing than before operation.

The plan tried in the first three grades of dividing the class so that each half of a room rotates between playground and class work resulted in a certain amount of gain in school progress, due to the fact that each teacher worked with smaller groups. It did not, however, eliminate repeaters such as have been described above, the majority of whom had been accorded the benefit of the scheme just outlined.

In view of these facts, it would seem that the success obtained with the experimental class was due not so much to the extra time spent on them as to the fact that this time was devoted to a study of the individual needs of these children. Here were eighteen school failures

according to the criterion of grading, and eighteen individuals whose pedagogical record and Binet-Simon findings would be credentials enough to admit them without question to that mysterious order called "the backward child." They could not be poured into the ordinary mold of school curriculum because of certain traits of personality that had to be discovered, understood, and wisely handled. The same psychobiological characteristics of shyness, indolence, fear, sensitiveness, daydreaming, etc., acted as conflicting factors in the Binet-Simon tests, obscuring the native capacity to such an extent that a difference of from one to three years appeared between the child's actual age and tested age.

A year of school training directed by an understanding of these characteristics has resulted in restandardization data with the Binet-Simon tests in June, 1921, that run parallel with the academic progress of these children as recorded in their grading reported above. The seven children who showed a difference of two years and over between their chronological age and mental age according to intelligence tests made in March, 1920, now show a mental level that coincides with their actual physical age.

And for those of us who examine such children, there is subject for reflection. Are we using the Binet-Simon scale as a yardstick to measure off lengths of intelligence, or are we using the tests as a help towards the sizing up of individual child problems? Are we confusing the term "mental age" with a diagnosis of the home situation in question? Do we see the personality with its setting of life story behind the intelligence level? These are possibilities of view that at times seem strangely remote from the productions of psychiatrists and practicing psychologists. As such, we should beware of agitating ourselves to decimal points of determination concerning qualifications for the groups of subnormal and dull, lest the object of our research becomes buried beneath the mound of his own case.

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NOTES ON NEW BOOKS

Studies in the Paleopathology of Egypt. By SIR MARC ARMAND RUFFER. Cloth, \$7.50. (University of Chicago Press, 1921.)

This is a collection of Sir Armand Ruffer's papers published after his death evidently with the loving aid of Lady Ruffer who worked over many incomplete manuscripts and brought together notes and literary references to complete them. The papers treat of an extraordinary material so romantic as to compel the interest of everyone to whom the names of Thutmose, Amenhotep and Rameses call up the ancient glory of Egypt.

The author had great opportunities for the study of the mummies of all the periods of Egyptian history and utilized them for the purpose of determining the character of the diseases from which the people suffered. This was relatively simple in the case of bony lesions, among which various forms of osteoarthritis deformans stand out prominently. Spondylitis with bony ankylosis of many vertebrae seems to have been common and periarticular exostosis hardly less so. Tuberculosis occurred and there is a case of Pott's disease with a psoas abscess, but there were no evidences of syphilis. The teeth furnish also much accurate information and it is readily seen that the ancient people took little care of them and that there were no efficient dentists. The teeth were often greatly worn and in an advanced state of caries with destructive abscesses which martyrised with toothache even such an exalted person as Amenhotep III.

It was found that the soft tissues of these mummies, although dried into unrecognisable form, could be softened and made pliable and even embedded in paraffin so that microscopical sections could be cut and stained. The tissues are by no means destroyed and cells can be recognised with their nuclei; muscle fibres with striations, elastic fibrils, areas of calcification and even bacteria and the eggs of *Bilharzia hæmatobia* can be clearly seen. The methods of embalming which are fully described were such as to destroy many of the tissues, often leaving others tightly wrapped round little statuettes which were placed in the body. The arteries were not used for injection of fluid, but were slashed away everywhere, and even most of the muscles were removed and replaced by all sorts of material. But although no connected examination of all the organs could be made, isolated observations of extreme arteriosclerosis, cirrhosis of the liver and renal disease were frequently possible.

Dwarfs, including some which were evidently chondro-dystrophic, and various malformed persons are unmistakably recorded in the ancient sculptures. The Queen of Punt looks as though she were the victim of congenital dislocation of the hips rather than rickets or elephantiasis.

There are various other studies such as that of a lower miocene crocodile with arthritis, an osteosarcoma of the pelvis, a variola-like eruption of the skin together with many non-descript ulcers and wounds, the traces of which remain and sometimes, as in the case of Sakounra, throw some light on history. These diseases are largely repeated in the case of the Copts, the Sudanese and the Nubians.

Most interesting are the studies of the Royal mummies which are, however, rather restricted owing to the divinity which hedges around these personages even now as they lie in the Cairo Museum. Everyone who has dwelt with pleasure upon the portrait busts of Ikhnaton, with their eager yet dreamy delicacy, must feel a shock of disappointment upon

learning that he was a ridiculously obese man even in a household where all the members were very stout. The rest seems not very important. Rameses II was bald and had blackheads on his forehead. Thutmose III and Amenhotep II had a macular eruption on their legs, and two or three queens were bald.

On the whole though, they were pretty well—all these royal people—and in spite of their consanguineous marriages they were preeminently strong, fertile, intelligent and vicious.

It is an extremely interesting account in spite of the rather wearisome repetitions, which are perhaps unavoidable in a collection of papers published at considerable intervals, and one is impressed by the infinite pains required, in handling these crumbling remains, to snatch from oblivion a few meagre hints of the discomforts and sufferings of these vivid people of thousands of years ago.

W. G. M.

The Care of the Eyes. By ROBERT HENRY ELLIOTT, M.D., B.S., Sc.D., F.R.C.S. (Eng.). (London, Henry Frowde and Hodder & Stoughton, 1921.)

This book is an elementary treatise, intended especially for nurses, on the subject of ophthalmic nursing and diseases of the eye. Of the three sections into which it is divided, the first deals with the special anatomy and the general and special therapeutic procedures used in ophthalmology. The second part contains a very brief description of the commoner diseases of the eye. The third, in the form of an appendix, is probably the most valuable section of the book. It illustrates the special instruments used in eye surgery, and gives excellent lists of those employed in the various operations.

Inasmuch as various ophthalmic clinics develop individual technique, nursing and therapeutic procedures within fairly wide general limits, the general value of any treatise, which outlines the technique and nursing procedures used in one individual clinic, will not be great. There are, however, many excellent chapters, and many essential points clearly made, which are of undoubted value. The second part of the book, dealing with diseases of the eye, is too brief. The appendix, describing instruments and lay-outs for various operations, is unusually complete. With such minor additions as any surgeon would make to suit his own individual preferences, this section will be of distinct value to the operating room nurse.

The book is well gotten up and quite readable.

A. C. W.

Obstetrics and Gynecology. Edited by JOHN S. FAIRBAIRN. Cloth, \$20.00. (London: Oxford University Press, January, 1921.)

The purpose of this book is evidently the very laudable one of bringing together in one volume the subjects of obstetrics and gynecology. This is in accord with the growing opinion that the two subjects are so closely related that the successful practice of one requires a thorough knowledge of the other. The arrangement of the subject matter is excellent and is proof that no sharp line of separation can be drawn between the two branches. The editor has wisely included many topics, such as public health and social and medico-legal problems, which are ignored in most texts.

Although this volume is the work of fifty-five authors, there is remarkably little overlapping of description and discussion. However, of necessity each topic is brief, and therein lies the chief weakness of the book. This brevity limits the mention and discussion of varying opinions to a minimum and, in many instances, to a more or less dogmatic expression of the author's individual views. This is particularly true of the sections on operative procedures.

It is unfortunate that the illustrations are so inadequate. More of them would be helpful, and many of those used are so reduced in size as markedly to limit their value.

J. W. H.

The Treatment of Common Female Ailments. By FREDERICK JOHN McCANN, M.B., M.R.C.P., F.R.C.S. (Edward Arnold & Co., London, 1922.) 145 pp. \$3.00.

This book of one hundred and forty-five pages has been written to guide the general practitioner in the general care of gynecological patients. The author deals with the various subjects in a concise and practical manner, giving many useful hints and prescriptions and covering a phase of gynecology which is too often overlooked by the modern specialist.

In spite of its merits, this little book is open to several criticisms. Like most works of this sort, it leaves one unsatisfied. The physician who is acquainted with the fundamental principles of gynecology usually does not need such an elementary text, and one who does not possess this acquaintance, needs far more. In the second place, we question the advisability of including in such a work descriptions of technical procedures such as the performance of operations, and the administration of radium, or the extended discussion of abstruse subjects like endocrinology, all of which are attempted in this manual. Furthermore, we feel that the author has been unfortunate in recommending certain procedures which seem to be antiquated, while failing to mention some of the noteworthy recent contributions. In general, although this book contains much that is practical and useful, it may prove disappointing to the practitioner who needs an up-to-date guide in the treatment of genito-urinary diseases in women.

L. R. W.

Syphilis and Its Treatment. WILFRED S. FOX, M.A., M.D. Cloth, \$9.00. (New York, Paul B. Hoeber, 1921.)

In this work intended for the use of medical students and practitioners of medicine, the author has limited himself chiefly to a discussion of the skin lesions of the disease. The illustrations consist of numerous water color and black and white drawings. There are few references to recent or remote literature, and the material represents the personal experiences of the author, treated in an informal way. Partly for this reason, but chiefly because the book presents only one side of the disease—and that perhaps the least important, from the standpoint of the patient—it falls far short of being a desirable text for students. Many writers on syphilis inadvertently (or purposely, as in this instance) fail to deal with syphilis as a disease affecting all portions of the bodily mechanism and stress only its manifestations as applied to certain organs or systems. This method of handling the subject, while satisfactory for those already expert in syphilology, is distinctly a faulty approach for the novice.

The text contains many rather dogmatic statements which either overlook the established facts or are not substantiated by them. For example, it is stated that except in monkeys, animal "inoculation experiments have been negative or doubtful. In rabbits there is a disease nearly related to it, if not identical, and Noguchi claims to have inoculated the testicles

of rabbits with syphilis." The existence of true rabbit syphilis has of course been thoroughly established by many observers, chief among whom are Neisser, Uhlenhuth and Mulzer, Nichols and Reasoner, and Brown and Pearce. The statement that mercury given by inunction is absorbed in considerable quantities through the lungs by inhalation is hardly in accord with known facts. The discussion of the paternal transmission of syphilis, will meet with many objections from the syphilologists of this country. The failure to mention routine spinal puncture as an indispensable accompaniment of the management of early syphilis constitutes a grave omission.

J. E. M.

Heart Disease and Pregnancy. By SIR JAMES MACKENZIE. (London, Henry Frowde, and Hodder & Stoughton, 1921.)

This small volume adds another to the list of works bearing the impress of the long clinical experience and research of the distinguished author, and has all the interest and charm one has learned to expect from the writings of Sir James Mackenzie.

While essentially a restatement of the newer knowledge of cardiac disease, as so often emphasized by the author, it constitutes an appeal for the application of this knowledge to all the problems relating to the question of marriage and the dangers involved in pregnancy and confinement for women the subjects of cardiac disease.

Written with the purpose of presenting his own views, based upon a long personal experience, to the obstetrician as the individual vitally concerned, one can not help feeling that the author has failed to appreciate, in a measure at least, the real extent to which the modern knowledge of cardiac disease has been made familiar to the obstetricians as well as to the profession generally. That just such a statement of the underlying principles involved will, however, prove of great value to many cannot be denied.

It is refreshing to read the author's description of the sequence of events in mitral stenosis of rheumatic origin and to get in his clearly put words the statement of the method for the correct evaluation of the physical signs in this condition as well as in aortic insufficiency. The chapter devoted to the neurotic heart covers this phase of the subject in a most satisfactory way.

Although lacking any exhaustive discussion of a number of points involving the pathological physiology concerned, and dealing chiefly with the clinical phases of the subject, there can be no question of the soundness of the views advanced.

A very complete index adds much to the value of the volume.

E. P. C.

Diseases of the Eye. By GEORGE E. DE SCHWEINITZ, 9th Edition. (Philadelphia and London, W. B. Saunders, 1921.)

That De Schweinitz' "Diseases of the Eye" has reached a ninth edition is a sufficient proof of its merit. In a book of this kind it is very difficult to meet the wants of everyone. Nevertheless, all in all, even the most critical should be satisfied. The revision of certain parts of the book and the foot-notes add materially to its value. The new edition is up-to-date, and includes a discussion of the more recent subjects in Ophthalmology—Localization and Organization of the Cortical Centers of Vision, The Dermic Grafts, The Epithelial Inlays, Jennings' Self Recording Tests for Color Blindness, Ophthalmoscopy with Red Free Light, Electric Desiccations, etc. Credit is given to other authors for their work or suggestions. In fine, Dr. De Schweinitz has given us another book which will render good service to the practising Ophthalmologist and the special student.

L. J. G.

Epidemiology and Public Health. By VICTOR C. VAUGHAN, HENRY F. VAUGHAN and GEORGE T. PALMER. Cloth, \$9.00. (St. Louis, C. V. Mosby Company, 1922.)

Progress in the study of infectious disease has been marked by a series of disappointments in the application of specific therapeutic measures following the early brilliant successes in diphtheria and meningitis. Today the immunologist concerns himself largely with matters only remotely related to the practical management of disease; the actual fight has been left to the epidemiologist and the public health worker. We lay more and more stress on the prevention of disease as a whole, as well as in the individual, and the recent blight, especially, cast by the influenza pandemic has impressed on those concerned with the health of the nation the essential importance of understanding and grappling with the conditions which underlie and promote the occurrence of such scourges.

It is with interest, therefore, that one approaches the new work on *Epidemiology and Public Health* by Victor C. Vaughan of which the first volume on *Respiratory Infections* is before us. After reading this section which contains an excellent summary, both of the historical development and of the contemporary status of epidemiological ideas, one feels that an era is closing. Despite the vast amount that has been accomplished, the hope of the future seems fully as great as the performance of the past. It becomes clear that with no more than our present knowledge the next influenza epidemic will blast us like the last; poliomyelitis and encephalitis are as baffling as ever from the standpoint of prevention, and in tuberculosis the ground is barely cleared for rational and concerted attack. The interesting fact that healthy carriers may harbor pneumococci or meningococci or diphtheria bacilli has as yet led to no fruitful practical method of checking epidemics due to these organisms. Epidemiology, in short, has thus far brought out mainly generalities about the spread and incidence of disease. In the succeeding era it may be hoped that the application, still incomplete and abortive, of these generalities will mature into a practical and certain control of the scourges yet unchecked.

In summary, Vaughan's book states the problem and brings it up to date, the dry facts being varied by sections in which the author's well-known ideas on the "protein poison" are elaborated.

A. L. B.

Human Parasitology. By DAMASO RIVAS, B.S.Boil., M.S., M.D., Ph.D. (1st edition, W. B. Saunders Company, Philadelphia and London, 1920. 715 pages, 422 figures, 18 plates.)

The reviewer purchased this book with a sense of comfort in having discovered the long needed comprehensive English treatise on parasitology. The appearance of the book is attractive and its size seemed designed to permit a certain degree of completeness. It was a pleasure to note in the preface that the author had "kept himself informed of changes in nomenclature" and was aware of the fact that "the ever changing nomenclature of parasitology is a continued source of embarrassment." This seemed to promise a discriminating criticism of the names given to parasites, and the adherence to valid terms.

This prefatory satisfaction, however, was not long sustained. In the text for Fig. 12, "linen threads" are noted in the diagram of the cell, and at this point the reader picks up a strand of error which runs through the fabric of the book. This, like the word "amiboid" on p. 606, may have been a typographical error. Throughout the book, however, there are many similar examples of inaccurate uses of words, uncritical compilations and contradictions. An insect is at one time described as the intermediate host, and at another the definitive host, of the same parasite. Although the author protests

his allegiance to the International Committee upon Zoological Nomenclature, he disregards the rules of that Committee, and indeed, revises the Latin language. He omits diphthongs not only when he uses the current simplified spelling of words derived from Latin, but also in proper generic and specific names. Thus *Amæba* becomes *Ameba*, and *Tænia* becomes *Tenia*—terms which have no validity under the rules of nomenclature. The embarrassing confusion of names given to one parasite is well illustrated by the different terms for the nematode "whip-worm," which the author scatters through the text. These are not grouped as synonyms under the valid designation, but occur as follows: *Trichocephalus*, p. 352, *Trichiuris*, p. 353, *Trichiuris trichiurus*, p. 358, *Trichiuris trichiuras*, p. 392, *Trichiuris trichiuris*, p. 449, *Trichocephalus trichiura*, p. 393, *Trichocephalus trichiuris*, p. 394, *Trichiuris trichiura*, p. 713.

Although the book bears the date 1920, there is little evidence to show that the author has kept up with recent work in this field. This is exhibited particularly in the section on the *Entamæba*. Dobell's work is not mentioned, while the fanciful conceptions of Schaudinn and Craig are repeated. *Entamæba histolytica* and *Entamæba tetragena* are described as distinct species, and their differences are set forth in a table. The use of emetine in the treatment of amebic dysentery is not mentioned.

The section on the *Flagellata* is confused and inadequate. The spirochætes, however, are included in this section without hesitation. Their inclusion leads to this statement on p. 220: "Noguchi and Pareja claim to have found a flagellate in the blood of yellow fever patients." Undoubtedly, *Leptospira icteroides* is referred to here. The statement is, to say the least, misleading, since it has not been determined whether the spirochætes are bacteria or protozoa.

The chapters on Helminthology are the best in the book. Here also contradictions occur in the text. In the table opposite p. 242 the genital pore of *Schistosoma* is said to be anterior to the ventral sucker, whereas on p. 259 it is said to be posterior to it.

The descriptions and figures of the insects are excellent in some respects, but inadequate for the differentiation of species. Several errors and misleading statements quickly strike the attention of the reader. On p. 552, *Glossina palpalis* is said to transmit *Trypanosoma brucei*, and on p. 514 it is mentioned without conviction that the "louse has been said to transmit the virus of typhus fever."

A great variety of fungi, and bacteria, together with the technic of mycology, bacteriology, microscopy, hematology, and serology are described in the final 100 pages of the book. The futility of this sort of compressed compilation is shown by the description of the microscope, which is contained in a paragraph of 22 lines. The yeast-like organism *Monilia* is classed with the *Hyphomycetes* along with *Actinomyces* (which is incorrectly called *Discomyces*). Many of the laboratory procedures described are antiquated, and the directions given are too sketchy to be of value.

There are many excellent pictures in the book, and also many which are drawn to such a small scale that the details of the structures supposed to be represented cannot be seen. These are, therefore, of no assistance in the elucidation of a text which is often obscure.

It would be unprofitable to continue to catalogue the errors found in this book. Much correct information is contained in it, and the book may serve as a means of orientation in parasitology. But its authority is destroyed by its errors and omissions. It disappoints expectations and does not supply the need of a comprehensive and accurate text-book in English on the subject of Parasitology.

S.B.-J.

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GENERALIZED MEGALOCARYOCYTIC REACTION TO SAPONIN POISONING

By J. FIRKET and E. S. CAMPOS

(From the Department of Pathology, The Johns Hopkins University)

Our interest in the subject of this investigation was aroused by the occurrence of a peculiar case of extreme anæmia in which there was complete aplasia of the bone marrow together with a new formation, in the liver and spleen, of myeloid tissue in which megalocaryocytes predominated. This case, in itself worthy of publication, we could not explain on the basis of anything that could be learned of the man's history, but in attempting to imitate it by any means in our power, we found that a very similar condition could be produced by saponin poisoning. Bunting, as we found later, had shown that saponin poisoning destroys many of the blood platelets and produces a multiplication in the bone marrow of the megalocaryocytes; these observations we have been able to confirm.

The case of anæmia was as follows: *Clinical History:* J. W., a healthy man, aged 40 years, began to feel weak during December, 1920. He continued working until

January 11th, 1921, but with increasing fatigue, weakness, dyspnœa and pain in the lumbar region. At about that time he developed œdema of the ankles and puffiness of the face. On January 13th the blood examination showed 1,744,000 R. B. C., 5,300 W. B. C., and 25% hæmoglobin. Two days later, during which he had been semicomatose, these figures were, 1,272,000 R. B. C., 4,200 W. B. C., and 17% hæmoglobin. The color index was 0.71. The blood picture showed especially anisocytosis, poikilocytosis, 48% neutrophilic polymorphonuclear leucocytes, 2.5% myelocytes and a very few normoblasts. Platelets were practically absent. Hæmolysis began at .48% sodium chloride and was complete at .32% sodium chloride. There were no other positive symptoms. Notwithstanding two transfusions of 500 c.c. each of citrated blood, which raised the red-cell count to 2,000,000 with 34% of hæmoglobin and 3,360 white cells, the patient

sank deeper into coma, the pulse became weaker and death followed on January 20th. Autopsy ten hours after death by Dr. Athens.

Anatomical diagnosis: Extreme anæmia, aplastic bone marrow; splenic tumor; focal necrosis of liver and spleen; extensive myeloid changes in liver and spleen; acute sero-fibrinous pericarditis and pleurisy; tuberculosis of mediastinal glands; caseous encapsulated nodule in left lung; catarrhal bronchitis.

Deposits of pigment were found in the liver cells, a slight hæmorrhage in the fundus of the eye and a complete aplasia of the marrow of the long bones, which was entirely fatty. Throughout the red pulp of the spleen and between the hepatic cells myeloid elements were present. It is very striking that, although in this myeloid tissue one finds normoblasts and myelocytes in fairly large abundance, proportionally the most numerous elements are megalocaryocytes. In other words, the number of megalocaryocytes is relatively larger than in any other myeloid tissue: this is especially in contrast with what is seen in other myeloid metaplasias of spleen and liver, as, for example, in cases of myeloid leukaemia. Some of these megalocaryocytes show a large, swollen, clear nucleus, with very little chromatin; others have a shrunken, deeply stained, almost pyknotic nucleus.

In the spleen there were some megalocaryocytes which had gone through the capillary system of the liver into the pulmonary circulation, where they were found in the sections of the lung.

As far as the diagnosis is concerned, the myeloid, or better, the megalocaryocytic reaction of the spleen and liver, the presence of focal necroses in the liver and spleen, the presence of iron pigmentation in the tissues and the absence of marked hæmorrhages, lead us to abandon the diagnosis of pure aplastic anæmia.

It seems probable that there was a poisoning of some sort and indeed the history records the fact that the patient was exposed four years before (but not since then) for about a year to benzol vapors and that the room in which he had worked lately was filled with fumes from soldering. It is obvious that the late effects of exposure to benzol vapor must be considered in our effort to explain the existence of complete aplasia of the bone marrow with the presence of myeloid tissue in other organs. Selling found that myeloid metaplasia occurred in the spleen after the cessation of the myelotoxic action of the poison and at a time when the aplastic bone marrow was regenerating itself. It was not possible to enter upon experiments to decide whether a stage in the recovery from benzol poisoning might be reached at which myeloid metaplasia of distant organs could coexist with a still completely aplastic bone marrow. This, as well as the question of the possible production of abundant megalocaryocytes in distant organs, remains to be determined.

Our interest took a different turn toward an effort to find some poison which would produce this condition.

Saponin is well known as a powerful hæmolytic agent, producing, as Foa states, a severe anæmia with complete aplasia of the bone marrow and simultaneously a myeloid metaplasia of the spleen. This then seemed the most suitable substance for our experiments.

Chemically the term saponin is applied to a number of glucosides which, according to Cushny, have many features in common both in their chemical properties and their pharmacological action. While the saponin bodies seem to have a localized destructive action upon the cells with which they come in contact, their effect is practically restricted to the blood and hæmopoietic organs when injected into the body.

Saponin had been used in the experimental production of anæmia in rabbits by Bunting in 1906. Later, as mentioned, in his paper of 1909 he records the fact that saponin destroys blood platelets as well as red corpuscles and stirs up in the bone marrow an excess production of megalocaryocytes. Isaac and Möckel in 1910 state that saponin destroys the bone marrow, leaving it aplastic and producing a myeloid metaplasia of the spleen resembling the condition to be found in the hypothetical "acute aleukæmic myeloid leukaemia."

The results of these experimenters led us to believe that by using saponin we might produce in rabbits a condition resembling that described in the fatal case in the human being.

The first experiments showed a conspicuous new formation of myeloid tissue in the liver and spleen with one feature which has not been observed by the other investigators, namely, the predominant new formation of megalocaryocytes in those organs. This becomes the chief point in our results, because on account of their function in forming blood platelets these cells assume a great importance in connection with the destructive action of saponin upon the platelets. Bunting had mentioned this rather specialized destructive action and we proceeded to study it in further detail and to consider the subsequent regenerative processes.

Before relating our experiments and observations, it is a pleasure to acknowledge our indebtedness for the opportunity for work afforded us in the laboratory of Dr. W. G. MacCallum.

MATERIAL AND METHODS

Saponin: The sample used was the same for all the experiments. It came from The Heyl Laboratories, Inc., Chicago. All the doses were weighed with the same balance and, on account of the instability of solutions of this glucoside, fresh saponin was dissolved for every experiment.

The physiological salt solution, used as solvent, was also made up with the same sample of chemically pure sodium chloride (Merck) previously dehydrated by one

hour's exposure at 150° C. The solution used was always at the strength of 0.9%.

All the animals used were of the same variety of Belgian hare, and none of them were very young. They were all submitted to the same conditions of food (wheat, water and occasionally fresh lettuce) and kept in the same room at a fairly constant temperature.

Each of the forty-five rabbits was given a number as soon as it was used once and this number was not changed afterwards.

All the injections were made intravenously after shaving and cleaning the region of the marginal vein of the ear. As the localized necrotic action of saponin, when used in relatively strong concentrations, was very marked, there usually occurred thrombosis of the veins of the ear with very often œdema, and later on, fibrosis of the cellular tissue surrounding the vein. This, in a few instances of long repeated injections obliged us to inject into the veins of the abdominal wall, without any other trouble than the same localized action just mentioned.

Autopsies. Each dead rabbit was submitted to autopsy. These were made most frequently immediately after death, as the rabbits were usually killed. Some rabbits found dead in their cages the next morning were examined as soon as possible, so that the tissues were not in any case exposed to post-mortem changes for a period of more than ten hours. All autopsies were performed according to the same method. The liver, spleen, bone marrow of the femur, tibia and humerus, adrenals, lungs and kidney were fixed. In many autopsies the lymph glands were also preserved.

Zenker's formol fluid (Helly's formula) was used as fixing agent; occasionally 10% formalin was used.

The organs, embedded in paraffin, were then sectioned, the sections being from 5 to 7.5 in thickness.

Longitudinal sections of the whole organs, as far as possible, and transverse sections of the bone marrow of each specimen, were made. This is important, as only a thorough examination of the marrow of all the long bones can give a true impression of the condition of this hemopoietic apparatus. A longitudinal section of the complete spleen was also cut in most cases.

The staining methods used were: Hæmatoxylin (Grübler's) and eosin; Wright's stain and the Poulenc "Pan-chrome de Laveran."

For blood examination and blood counting the usual routine methods were employed. The same pipette and counting-chamber (Thoma-Zeiss) were used for all the counts of one animal. The diluting fluid for red corpuscles was 0.9% salt solution and the special fluid used for platelets (see below); for the white cells, Türk's

fluid. As soon as the sample of blood was diluted, the pipette was shaken in an electric shaker for from five to ten minutes to avoid any agglutination and precipitation.

The hæmoglobin estimation was made always with the same Gowers-Sahli hæmoglobinometer.

In order to make the report of our results clear, we shall proceed in the following manner: the experiments will be selected according to the problem to which they are related; by that means we shall deal first with the question of blood destruction which has led us to study (1) the resistance of the red cells to saponin; (2) the specific action of saponin on blood platelets *in vivo* and *in vitro*; after this the process of regeneration will be described and with it the most appropriate protocols of experiments and autopsy records.

Finally, we shall have to consider the question of the mechanism which leads to the so-called "aplasia" of the bone marrow after injections of saponin.

STUDIES OF THE RESISTANCE OF RED CELLS TO SAPONIN

When we began systematically our study of the action of saponin, we realized that in order to come to an understanding of the blood curve, we had to study not only the process of regeneration but also the process of destruction. Indeed, there was a possibility that the resistance of the erythrocytes of normal rabbits might change after saponin injection, making difficult our understanding of the blood curve.

Realizing this, in a first series of experiments we investigated the resistance of the red blood cells of the normal rabbits to our sample of saponin. After we had found this we tested the resistance of the red blood cells of the same rabbits after they had been injected with saponin.

In a third series of experiments, we investigated the same property of the blood in splenectomised rabbits. This was done, because it had been shown by Pearce and others, in their experiments on splenectomised dogs, that there is, after splenectomy, an increased resistance of the red cells to hypotonic salt-solutions, hæmolytic serum, saponin, and other injurious substances.

Technique.—Only washed blood-cells and not the blood *in toto* were used for the hæmolytic test. The blood was taken through a puncture of the marginal vein of the ear and allowed to drop freely into a 1 per cent solution of sodium citrate. The suspension was then centrifuged, the plasma removed by means of a pipette, and the red corpuscles washed three times with a 0.9% NaCl solution. A suspension of washed red cells of about 3% was then made in the same physiological solution.

Saponin was prepared in the same salt solution at the strength of .25 mmgr. saponin for 1 cc. of physiological salt solution. This mother solution was diluted with physiological salt solution as follows:

Tube No.	Sol. of Saponin .25 mmgr. for 1 cc. salt sol. .9%	0.9% Salt solution
10	.10 cc.	.90 cc.
11	.09 cc.	.91 cc.
12	.08 cc.	.92 cc.
13	.07 cc.	.93 cc.
14	.06 cc.	.94 cc.
15	.05 cc.	.95 cc.
16	.04 cc.	.96 cc.
17	.03 cc.	.97 cc.
18	.02 cc.	.98 cc.
19	.01 cc.	.99 cc.
20	0.00 cc.	1.00 cc.

To each tube of this system were added, with the same pipette, two drops of the suspension of red corpuscles. The hemolytic system was placed in the water-bath at 38° C. for four hours, during which it was shaken several times. When taken out of the water-bath, the readings were made after half an hour of sedimentation. In describing our results, we shall refer to the tube numbers given in the above table.

Results of the Hemolytic Tests with Saponin in Normal Rabbits

By normal, we mean the rabbits having not yet received an injection of saponin and those which did not present at autopsy any lesions of coccidiosis or other parasitic infection.

No. of rabbit	Date	Hæmol. begins in tube	Hæmol. complete in tube
23	May 21	13	12
24	May 21	13	12
30	May 21	13	12
30	May 28	13	12
30	May 31	13	11
38	June 30	13	12

These results indicate that the erythrocytes of different rabbits differ only slightly as regards their resistance to saponin. It also shows that resistance remains fairly constant in the same rabbit (Rabbit No. 30).

Results of the hæmolytic tests with saponin in rabbits having received intravenous injections of saponin.

No. of rabbit	Before Injection		After Injection		No. of injections received
	Hæmol. begins in tube	Hæmol. complete in tube	Hæmol. begins in tube	Hæmol. complete in tube	
21	13	12	13	12	6 inj. 4.2 mg. in 7 days
24	13	12	13	12	3 inj. 5.8 mg. in 4 days
33			13	11	11 inj. 1 mg. in 15 days
25			13	12	10 inj. 5.8 mg. in 10 days

Hæmolytic Tests with Saponin in Splenectomized Rabbits

The rabbits of the following table had not received, when the test was made, any injection of saponin.

No. of Rabbits	Condition	Hæmolysis begins in tube	Hæmolysis complete in tube
24	Normal	13	12
29	Splenectomized 2 days ago	14	12
27	" 7 days ago	13	12
8	" 7 days ago	13	12
19	" 11 days ago	13	11
29	Splenectomized 12 days ago	13	11
27	" 15 days ago	13	11
19	" 18 days ago	14	12
19	Splenectomized 48 days ago	14	11
38	Normal	13	12

Although Rabbit No. 19 did not show any increased resistance to saponin, it had shown, six days before the last test, an increased resistance of its red cells to hypotonic salt solutions, as can be seen from the following table. This system was prepared so that—
Tube 1 contained 20 drops of physiological salt-solution, Tube 2 contained 19 drops of physiological salt-solution plus 1 drop of distilled water, Tube 3 contained 18 drops of physiological salt solution plus 2 drops of distilled water, Tube 20 contained 1 drop of physiological salt solution plus 19 drops of distilled water.
To each tube of this system two drops of a 3% suspension of the washed red cells were added.

Hæmolytic Test with Hypotonic Salt Solution in a Splenectomized Rabbit

No. of rabbits	Condition	Hæmolysis begins at tube	Hæmolysis complete in tube
38	Normal	9	14
37	Normal	9	14
19	Splenectomized 42 days ago	11	15

In summing up these results we are permitted to draw only the following conclusions:

(1) After intravenous injections of saponin in rabbits, the resistance of red cells to this drug does not show any marked change.

(2) The red cells of splenectomized rabbits are not different in their resistance to saponin from those of normal rabbits, although they are more resistant to hypotonic salt solution.

This is not completely in accord with what Pearce found in his splenectomized dogs in which increased resistance of red corpuscles to saponin as well as to hypotonic solutions and other hemolytic substances occurred. They do not differ, however, from the observations of McNeil in man.

Although it is not our purpose to discuss at length the question of hæmolysis by saponin, this part of our results

convinced us that, as far as the corpuscles alone were concerned, the destructive power of saponin remains fairly constant when used in the same amounts and the same dilutions. We know, however, that the action of saponin *in vivo* is far more complex than that shown in a test-tube with washed corpuscles. Ranson found that cholesterol and lecithins, in salt solution, have a protective power against hemolysis by saponin. Clark and Evans have recently shown that normal human serum shows a protective power against hemolysis by saponin far more active than the protective power of cholesterol and lecithin; they were, however, unable to tell to what mechanism or substance this normally present protective power of serum against hemolysis by saponin was due.

We have not made any study along these lines and have not investigated the chemistry of the blood of our rabbits before injection. We had the impression, however, that in our series of rabbits two pregnant animals showed a greater resistance to saponin. Receiving the same amount of saponin intravenously, they became less rapidly anemic, and this was due certainly to a diminished destruction of corpuscles, because the hæmopoietic reaction, found at autopsy, was less marked in these animals than in the controls. For this reason, and to maintain more constant conditions, we used later only male rabbits which, being all fed in the same manner, would have probably a more constant amount of lipoids in their blood.

ACTION OF SAPONIN ON BLOOD PLATELETS

As we found, in our first rabbits injected with saponin, the liver and spleen filled with megalocaryocytes, we were naturally forced to consider the action of this drug on platelets. Indeed, most of the hematologists in Europe as in this country, agree at present with Wright's doctrine of the megalocaryocytic origin of blood platelets (Naegeli, Schridde, Cesaris-Demel, Ferrara, Aschoff, Ogata, Downey, Klein, Foa, Brown, Lesourd and Pagniez, Di Guglielmo, and others), although some Italian investigators, for example, Pianese and Perroncito, have rejected it recently.

Consequently we were inclined to think that a careful investigation of the platelet curve of our injected rabbits would be of some use.

Technique. In order to avoid, as far as possible, any causes of error, we were always careful to draw the sample of blood from the first drop of blood arriving freely after the puncture of the marginal vein of the ear of the rabbit. In drawing the blood into the red counting pipette, the tip of the pipette was held in the middle of this drop and did not touch the lips of the vascular wound. Then it was rapidly diluted in the pipette with physiological salt solution or more often with the following fluid: 30 cc. of a 3% solution of sodium citrate in distilled water and 1 c.c. of a 1:150 brilliant-cresyl-blue solution in

distilled water, the two fluids being mixed and filtered before use.

As soon as the pipette was filled with blood, it was shaken in the electrical shaker. If this is done rapidly, one succeeds almost always in avoiding any agglutination of platelets. The very few samples in which agglutination was observed were discarded as not reliable for accurate counting. After ten minutes' shaking, the drop for examination was allowed to deposit for twenty minutes, which is the time required for a complete sedimentation of the platelets. Let us add that all counts have been made with the same pipette and same chamber, by the same observer, and were often controlled by the other.

The following table shows the effect upon the platelets of the injection of large doses of saponin.

TABLE I.

Reduction of platelet-count after injection of saponin.

Rabbit	Date	Time	Saponin injected	Erythrocytes per cu. mm.	Platelets per cu. mm.
40	June 29—				
		9 a.m.	—	7,776,000	752,000
		9:45 a.m.	15 mgm. in 15 cc. phys. salt solution		
		10:15 a.m.	—	6,400,000	496,000
		2 p.m.	—	5,850,000	200,000
		4:15 p.m.	—	5,800,000	160,000

On June 30th the animal was found dead.

41	July 1—				
		9 a.m.	—	6,100,000	532,000
		10:30 a.m.	15 mgm. in 7.5 cc. phys. salt solution		
		12:30 p.m.	—	4,800,000	320,000
		2:30 p.m.	—	4,936,000	290,000
		4:30 p.m.	—	5,008,000	272,000

On July 2nd the animal was found dead.

Similar observations were frequently made during our experiments. They show plainly that there is a diminution of the number of platelets in the circulating blood, following an injection of saponin.

We had to keep in mind, however, that several substances have been used to diminish temporarily the blood platelets of the circulating blood. Quite recently Roskam succeeded in doing so in dogs, with injections of gelatin. While one does not know exactly what becomes of these platelets, one might wonder if they were not stored up in the visceral circulation.

Two rabbits were used to answer partly this question as regards saponin. After injection of saponin they were anesthetized with ether, and laparotomized; simultaneously samples of blood were taken by puncture of the vein of the ear and of the portal or splenic vein.

These experiments, while very concordant, show that the number of platelets circulating in the blood of the portal system is not higher than the number of platelets

TABLE II.
Platelet counts in peripheral and visceral blood.

Rabbit	Date	Time	Saponin injected	Peripheral blood vein of		Visceral blood Portal and Splenic veins	
				Erythrocytes per cu.m.	Platelets per cu.m.	Erythrocytes per cu.m.	Platelets per cu.m.
39	June 28—	a.m.	—	6,576,000	670,000		
		1:15 p.m.	25 mgm. in 20 cc. phys. salt solution				
		2:45 p.m.	—	6,070,000	360,000	Portal Vein	
		4:45 p.m.	—	4,800,000	328,000	4,832,000	304,000
44	July 11—	8:30 a.m.	—	6,626,000	782,000		
		9:15 a.m.	15 mgm. in 5 cc. phys. salt solution				
		11:20 a.m.	—	6,400,000	342,000		
		1:15 p.m.	—	6,144,000	280,000	Splenic Vein	
		4 p.m.	—	6,648,000	220,000		
		5 p.m.	—			4,996,000	200,000

in the peripheral blood. They do not allow us, however, to discard the hypothesis that blood platelets are retained in the capillaries of an organ or even of the whole body.

A real proof that we are dealing with a destruction of platelets would be to obtain this destruction *in vitro*, and

TABLE III.
Action of saponin *in vitro*.

Rabbit	I. Saponin solution: 0.25 mgm. per 1 cc. 0.9% NaCl	II. 0.9% NaCl solution	Correspond- ing tube in hemolytic system	Platelets per cu.mm.	
42	cc.	cc.			
		1		428,000	
	0.035	0.965		262,000	
	0.04	0.96	16	260,000	
	0.045	0.955		240,000	
	0.05	0.95	15	104,000	
	0.055	0.945		100,000	
	0.06	0.94	14	36,000	
	0.07	0.93	13	48,000	
	0.075	0.925		40,000	
44		II. Sodium citrate and brilliant cresyl blue		Platelets	Erythrocytes
		1 cc.		420,000	7,936,000
	0.06 cc.	0.94 cc.	14	148,000	7,736,000
	0.07 cc.	0.93 cc.	13	172,000	6,584,000
		1 cc.		488,000	6,080,000
Another sample of blood, other pipette and chamber	0.06 cc.	0.94 cc.	14	196,000	5,584,000
Another sample of blood		1 cc.		496,000	6,968,000
	0.08 cc.	0.92 cc.	12	168,000	6,112,000
Another sample of blood		1 cc.		364,000	5,824,000
	0.09 cc.	0.91 cc.	11	176,000	5,808,000

a series of experiments was planned to see if this could be done.

Technique: Several samples of blood, drawn from the same drop, were taken in several pipettes; one with ordinary 0.9% salt solution or with the fluid used for platelets (see above); others with physiological salt solution to which had been added a certain amount of a solution of saponin containing .25 mg. to 1 c.c. of salt solution. These last diluting fluids were measured so that hemolysis could not be obtained at all or could be obtained only after a long exposure. The strength of these dilutions were known by our previous hemolytic tests. When all the pipettes were filled with these special diluting fluids, they were shaken in the electrical shaker. As twenty minutes were required for the sedimentation of the drop in the counting chamber before any counts of platelets were made, the platelets were exposed to the action of saponin, *in vitro*, for about 30 minutes.

Of course, none of the rabbits used in these experiments had received previously any injection of saponin.

This demonstrates that saponin in physiological salt solution, in a dilution which hemolyzes red cells slowly or not at all, destroys many of the platelets. It is probable, therefore, that the platelets do not all show *in vitro* the same resistance to hemolytic agents, because some survive while others are destroyed just as in the case of the red corpuscles, for it is well known that young red cells, more especially nucleated red cells, have a higher power of resistance than mature erythrocytes.

While Bunting deserves entire credit for the discovery of this action of saponin, we think that a report of our experiments may be useful, because they seem to meet, more clearly than Bunting's, the objections which may be raised against the statement that saponin destroys the platelets. Several investigators have attempted to withdraw all the platelets from the circulating blood by means of injections of such substances as gelatin. This is effective in causing their disappearance, but we do not know how it acts nor whether the platelets are really destroyed. The actual destruction of platelets would stimulate their specific regeneration in the hemopoietic organs, while this would probably not follow the action of any agent which only temporarily withdraws platelets from the peripheral blood.

Process of Regeneration

Our rabbits injected with saponin did not all show the same clinical picture. It is well known that besides a toxic action, not always measurable and leaving no lesions, saponin destroys the corpuscles in the circulating blood, and, also, as Bunting and the writers have shown, destroys the blood platelets. Thus the effect of saponin, like that of any other poison, is subjected to many factors which are probably not all known and not measurable, depending on the individual resistance, the concentration of the dose, its excretion from the body, *et cetera*.

But as far as its action on the blood curve is concerned, we had an impression that in general our rabbits behaved in two different ways; some receiving doses of saponin became rapidly anemic, lost weight and appetite and died after a rather short time with a high degree of anemia (rabbits 3, 5, 19, 38); others, receiving somewhat smaller doses (per body weight) or the same doses but less often repeated, showed in the beginning also anemia, loss of weight and appetite. They then entered a period where the same doses of poison did not seem to have the same effect any more on their blood picture; the red blood cell count became higher again; the rabbits, more lively, seemed to have come to a stage of equilibrium in which their hæmopoietic organs were able to restore daily the blood elements destroyed (rabbits 11, 12, and others). On the contrary, the rabbits of the first category, while their hæmopoietic organs showed a tremendous attempt to repair their losses, were unable to reach before death this stage of equilibrium.

In the rabbits which had received injections of saponin during long periods, there was an adaptation of the activity of the hæmopoietic organs to the losses of blood, and this was made still more clear in the rabbits which did not receive regularly the same dose. These, while running a long course, became irregularly anemic, but never were able to reach the stage of equilibrium above mentioned (rabbits 1 and 4).

The rabbits of another group received a dose strong enough to kill them in less than twenty-four hours, so that their hæmopoietic organs had not had time to show any tendency to repair the loss of blood (rabbits 39, 40, 41). Consequently, we shall present separately the records of these three groups.

Rabbits 39, 40 and 41: All these rabbits were injected with large doses of saponin from 15 mgr. to 30 mgr. All of them died in less than twenty-four hours after the injection. The autopsy findings were all alike: liver and spleen normal; hyperemia of the bone marrow with numerous diffuse and small hemorrhages; no increase of cellular activity in the bone marrow but a large number of megalocaryocytes. While the spleen, the liver and other organs show no pathological changes, the bone marrow even so shortly after the injection (four or five hours for rabbit 39) shows a marked dilatation of its capillaries filled with blood and diffuse hemorrhages.

We shall describe the protocols of three rabbits of the second group which had shown tremendous restorative efforts of the hæmopoietic organs.

AUTOPSY (performed immediately): Bone marrow dark red. Microscopically: *Spleen.* Tremendous myeloid metaplasia with normoblasts, myelocytes and especially megalocaryocytes (see Fig. No. 1). These are proportionally more abundant than in any other myeloid tissue; this is so well marked that one seems to deal with a genuine megalocaryocytic reaction. These megalocaryocytes

PROTOCOL OF RABBIT No. 3

(Old, long hair)

Date	Time	Saponin injected intravenously	Erythrocytes	Leucocytes	Hemoglobin	Observations
Mar. 10—						
	3 p.m.	—	4,084,000	11,400	104%	
	4 p.m.	5 mgm. in 2.5 cc. phys. salt sol.	—	—	—	
Mar. 11—						
	3 p.m.	—	2,416,000	44,000	—	
	4 p.m.	5 mgm. in 2.5 cc. phys. salt sol.	—	—	—	
Mar. 12—						
	1 p.m.	4 mgm. in 2 cc. phys. salt sol.	—	—	—	
Mar. 14—						
	11 a.m.	—	400,00	10,200	85%	Paraplegia of hind legs
Mar. 14—						
	12 a.m.	—	—	—	—	The animal dies

show numerous figures of Wright and marked phagocytosis of polymorphonuclear leucocytes with pseudo-eosinophilic granules and small lymphocytes. All the myelocytes found have pseudo-eosinophilic granulations; no basophilic myelocytes are seen. Many macrophages containing granules of pigment exist throughout the splenic pulp. Many free megalocaryocytes, myelocytes and normoblasts are in the large splenic sinuses and splenic veins.

Liver: Many megalocaryocytes in the capillaries (see Figs. 2 and 3). Most of them probably come from the spleen and stop in the hepatic circulation. Some may be formed in the liver itself. Also, in the hepatic circulation there are myelocytes and normoblasts which have surely the same origin as the megalocaryocytes.

Lung: Myelocytes and megalocaryocytes in the capillaries and in the pulmonary vessels. The megalocaryocytes are almost devoid of cytoplasm.

Adrenal: A few megalocaryocytes in the capillaries (see Fig. 4).

Kidney: Normal.

Bone marrow: Hyperplasia with many normoblasts and erythrocytes and many megalocaryocytes, which show a great tendency to phagocytosis. Hyperæmia and hemorrhages.

Rabbit No. 5. This rabbit showed exactly the same clinical picture; became rapidly anæmic, lost 300 grams and died after five days.

Two and three days after the beginning of the injection, the number of normoblasts in the circulating blood was very high. The autopsy findings are exactly the same as for Rabbit No. 3. Abundant hemorrhages in the bone marrow.

These two typical autopsies show how rapidly the rabbits become anæmic during the first days after injection. They died without having reached any equilibrium be-

tween the losses of blood-elements and the repairing process, although there is a great activity in the hæmopoietic organs (bone-marrow, spleen and liver). Megalocaryocytes are very abundant in all these organs. Hæmorrhages of the bone-marrow are more numerous than they were in the first group described.

It is interesting to notice that, even when the anæmia occurs less rapidly, the hæmopoietic reaction to saponin injections seems, in animals killed at the same period, quite as marked. This is shown by the following record:

PROTOCOL OF RABBIT No. 24

(Weight 1940 grams; female, pregnant)

Date	Time	Saponin injected intravenously; 4 mgm. per kilogram of body weight	Erythrocytes	Leucocytes	Hæmoglobin per cent
May 17—	—	—	7,168,000	13,200	101
May 22—	10 a.m.	5.8 mgm. in 1.41 cc. phys. salt sol.	—	—	—
May 23—	10 a.m.	Similar injection	—	—	—
May 24—	10 a.m.	Similar injection	—	—	—
May 25—	10 a.m.	—	5,568,000	15,200	71

The rabbit is killed at 12 m., being in excellent condition.

AUTOPSY: *Spleen*—numerous megalocaryocytes and several myelocytes.

Liver: Megalocaryocytes, showing phagocytosis, and very few myelocytes in the capillaries.

Lung: Megalocaryocytes, in the capillaries, almost deprived of their cytoplasm.

Bone marrow: Hyperæmia; hyperplasia with many megalocaryocytes.

Lymph gland: Normal; no megalocaryocytes or myelocytes.

Adrenal: No megalocaryocytes.

While the autopsy findings of the three last records are almost the same, it must be noticed that Rabbit No. 24 became much less anæmic than Rabbits 3 and 5; this may have occurred because it was pregnant and in this condition, on account of the increased cholesterinæmia, the protective power of the blood serum against hæmolysis by saponin may be increased.

Before we describe the case of a rabbit, in which an equilibrium between the destructive and repairing processes is reached, we shall present a case where, although the animal remained living for about twenty days, the anæmia was irregularly progressive and no equilibrium

was ever reached. This was due to the irregularity of the amount injected.

PROTOCOL OF RABBIT No. 4

(Weight 2375 grams on March 16th)

Date	Time	Saponin injected intravenously	Erythrocytes per cu.mm.	Normoblasts per cu.mm.	Leucocytes per cu.mm.	globulin per cent
March 16	10 a.m.	6 mgm. in 2 cc. phys. salt sol.	5,600,000	—	12,000	—
March 17	10 a.m.	Similar injection	3,348,000	376	37,600	85
March 18	10 a.m.	Similar injection	2,357,000	—	43,000	78
March 19	10 a.m.	Similar injection	2,048,000	2480	24,800	70
March 21	10 a.m.	7.5 mgm. in 2.5 cc. phys. salt sol.	816,000	327	10,900	60
March 22	12 a.m.	Similar injection	720,000	792	13,200	60
March 23	11 a.m.	Similar injection	690,000	1026	18,000	52
March 26	10 a.m.	Similar injection	708,000	790	15,000	40
March 29	10 a.m.	6 mgm. in 2 cc. phys. salt sol.	972,000	455	9,100	50
March 30	11 a.m.	8 mgm. in 2 cc. phys. salt sol.	786,000	315	10,500	48
Mar. 31-12	—	10 mgm. in 2.5 cc. phys. salt sol.	—	131	—	—
April 1	—	—	600,000	82	8,200	45
Weight 1790 gms.	11 a.m.	Similar injection	—	—	—	—
April 2	10 a.m.	Similar injection	436,000	968	8,600	45
April 4	11 a.m.	Similar injection	760,000	396	13,200	47

On April 5th the animal was found dead.

AUTOPSY: Weight—1320 grams. The inferior part of the bone marrow of the long bones is red and the superior part is pinkish gray. Liver and spleen enlarged. Some lymph glands also show a slight increase in size.

Microscopically: Liver—necrotic foci; slight myeloid reaction and exaggeration of the connective tissue.

Spleen: A greater myeloid reaction with numerous megalocaryocytes.

Adrenals: Some megalocaryocytes situated between the cortical and chromaffine tissue.

Bone marrow: Well defined focus of necrosis resembling a fresh infarct. Hyperplasia of moderate degree.

We shall describe now two rabbits presenting the typical history of those where an equilibrium between the effects of blood destruction and blood repair is reached. The second rabbit described was splenectomised and shows a very interesting blood regeneration.

PROTOCOL OF RABBIT No. 12
(Weight on April 29th—2340 grams)

Date 1921	Time	Saponin injected intravenously 4 mgm. for body weight	Platelets per cu.mm.	Erythrocytes per cu.mm.	Leuco- cytes per cu.mm.	Hæmo- globin per cent
April 23	—	—	—	6,384,000	5,900	102
April 29	—	—	—	5,632,000	9,200	93
April 30	—	—	580,000	—	—	—
	2 p.m.	5.6 mgm. in 1.4 cc. phys. salt sol.	—	—	—	—
May 2	—	—	112,000	2,567,000	30,000	71
	10 a.m.	Similar injection	—	—	—	—
May 3,	—	—	—	—	—	—
	4 p.m.	Similar injection	—	—	—	—
May 4	—	—	250,000	1,392,000	8,000	65
May 5	—	Similar injection	—	—	—	—
May 6	—	—	—	2,400,000	6,200	50
	12 a.m.	Similar injection	—	—	—	—
May 9	—	—	396,000	3,328,000	4,400	50
	12 a.m.	Similar injection	—	—	—	—
May 10	—	—	—	—	—	—
	12 a.m.	Similar injection	—	—	—	65
May 11	—	—	386,000	3,936,000	10,000	65
	12 a.m.	Similar injection	—	—	—	—

On May 11, at 4 p.m., the animal died.

AUTOPSY: The bone marrow is firm, pinkish-gray in some places and grayish-white in others.

Microscopically: The *spleen* shows numerous megalocaryocytes and numerous macrophages loaded with pigment.

The *liver* shows pigmentation of the hepatic cells and a few megalocaryocytes.

The *bone-marrow* shows hyperplasia, hyperæmia, hæmorrhages (see Fig. No. 5) and foci of necrosis where there is a beginning of organization.

In the *lungs* there are some megalocaryocytes almost without cytoplasm.

PROTOCOL OF RABBIT No. 11

At 3 p.m. the animal was in very good condition. Then it was killed as a control for Rabbit No. 12 whose spleen had not been removed.

AUTOPSY: Liver: A few megalocaryocytes, in the capillaries; almost no myelocytes. Pigment in the hepatic cells.

Adrenal: Normal.

Lymph nodes: Several lymph nodes show myeloid reaction with numerous megalocaryocytes, several showing phagocytoses. (See Fig. 6.)

Lung: A few foci of hæmorrhages.

Bone marrow: Hyperplastic almost everywhere, with all kinds of myeloid cells (myelocytes, normoblasts, but more especially, many megalocaryocytes). Several megalocaryocytes show figures of Wright and phagocytosis. Other portions of the bone marrow are not very hyperplastic and remain fatty; even here, however, the most

Date 1921	Time	Saponin injected	Hæmo- globin per cent	Platelets per cu.mm.	Erythrocytes per cu.mm.	Leuco- cytes per cu.mm.	Observ- ations
April 22	—	—	104	—	6,992,000	11,000	—
April 23	—	—	—	—	—	—	Spleen rem'd
April 27	—	—	105	—	8,096,000	6,900	—
April 30	—	—	103	840,000	7,752,000	—	wgt. 1700 gms
	2 p.m.	4 mgm. in 1 cc. phys. salt solution	—	—	—	—	—
May 2	—	—	95	400,000	4,256,000	12,000	—
May 3	—	—	—	—	—	—	—
	4 p.m.	Sim. inj.	—	—	—	—	—
May 4	—	—	90	192,000	3,200,000	24,000	—
	3 p.m.	Sim. inj.	—	—	—	—	—
May 5	—	Sim. inj.	—	—	—	—	—
May 6	—	—	72	—	3,232,000	16,000	—
	2 p.m.	Sim. inj.	—	—	—	—	—
May 9	—	—	90	—	4,800,000	11,600	—
	2 p.m.	Sim. inj.	—	—	—	—	—
May 10	—	Sim. inj.	—	—	—	—	—
May 11	—	—	96	—	5,560,000	6,000	—
	2 p.m.	Sim. inj.	—	—	—	—	—
May 12	—	—	—	—	—	—	—
May 13	—	—	100	—	6,008,000	6,400	—
	2 p.m.	Sim. inj.	—	—	—	—	—
May 14	—	Sim. inj.	—	—	—	—	—
May 15	—	Sim. inj.	—	—	—	—	—
May 16	—	Sim. inj.	—	640,000	—	—	—
May 17	—	—	—	—	7,392,000	—	—
	1 p.m.	Sim. inj.	—	—	—	—	—

numerous cells, among the fat cells, are megalocaryocytes. Scattered throughout the organ are numerous foci of hæmorrhages; other portions show young granulation tissue with fibroblasts beginning to elaborate connective fibers.

SUMMARY AND DISCUSSION

We have studied rabbits which had been injected with large doses of saponin, others with small doses, the doses remaining the same or being changed at each injection. We have studied rabbits, so treated, during a few hours, others during a few days, still others during a few weeks (Rabbit No. 33, one month). Some rabbits were splenectomized. We have never found an aplasia of the bone-marrow.

On the contrary, the preceding protocols demonstrate that while on the day of the first injection there is no appreciable hæmopoietic activity, a few days later (3, 4 or 6 days) the bone-marrow is hyperplastic and that also in the spleen and the liver a metaplasia of myeloid elements takes place. Let us insist on the fact that in adult rabbits the presence of myeloid elements between the fat cells does not mean necessarily hyperplasia, as it would in man. Normally the adult rabbit has a bone marrow with a fairly large amount of myeloid cells, as Roger and Josué had shown some time ago and as everyone familiar with blood formation in rabbits knows. It is necessary to compare almost the entire bone marrow of the experimental rabbit with that of the normal to reach a decision as to the presence or absence of hyperplasia.

This being known, we observed, however, on the third, fourth and fifth days following the injection, a definite hyperplasia of the marrow, but a disturbing factor to this hyperplasia was always present: hæmorrhages occurred following a marked hyperæmia of all the capillaries. (See Fig. 7). These hæmorrhages in the bone marrow are the only lesion found during the first day after injection of saponin and this leads us to believe that at the start there is an impairment in the circulation and consequently in the function of the bone marrow. This is probably the reason why myeloid metaplasia occurs so soon in other organs (spleen, liver, and in certain conditions in lymph-nodes). In order to repair the losses of blood-cells, a complementary blood-forming tissue is needed, as the normal myeloid tissue is unable to develop with full strength.

Isaac and Möckel raised the question whether such a condition, which they also saw in experimental poisoning by saponin, could not be compared to myeloid aleukæmic leukaemia. In fact, when we saw the tremendous metaplasia described in Rabbit No. 3, for example, without much evidence of myeloid elements in the circulating blood, the same comparison came into our minds. This seems, however, less reasonable than the explanation of a vicarious blood formation due to functionally impaired bone marrow. Indeed, in using saponin we produce above all an erythropathia. As saponin has practically no action on leucocytes, it seems improbable that the reaction to such a poison would resemble one of the diseases involving especially white cells.

The myeloid metaplasia, taking place outside of the bone marrow, is strikingly a reaction to the loss of blood platelets and red cells which are the elements destroyed. Megalocaryocytes and normoblasts are proportionally the most numerous elements of that myeloplastic tissue. Of course, there are some myelocytes, but their number is conspicuously low.

Megalocaryocytic reaction. Such a statement cannot be made without considering somewhat carefully the question of megalocaryocytic reaction. Dominici, Pianese and others, studying many sections of the spleen and liver of several normal animals, found in all of them a few megalocaryocytes. Some of the sections of these organs, in our normal rabbits, show also occasionally a megalocaryocyte. We speak of megalocaryocytic reaction only when this number is markedly increased (see figures). That this reaction is markedly increased in relation to the destruction of platelets is probable, although we do not find ourselves able to say that no other platelet-repairing mechanism than this exists. Brown and Ferrata have shown that monocytes or cells resembling them could form platelets (monocytoid cells of Ferrata). Bunting is of the opinion that even lymphocytes give rise to blood platelets.

Although we have not in mind to study especially the morphology of megalocaryocytes, we have observed during our experiments the following types of these cells:

Types of megalocaryocytes: The megalocaryocytes found in the bone marrow, spleen, liver and other organs of the rabbits injected with saponin, vary in appearance with respect to their size, shape, structure of the cytoplasm and of the nucleus (see Figs. 8 and 9). However, megalocaryocytes described as belonging to different types of cells, may well have been really the result of sections of the same kind of cell made at different levels. This fact must be kept in mind, especially when we are dealing with megalocaryocytes of rabbits, which are far larger than the human megalocaryocytes and in which the nuclei are more complicated. Carnegie Dickson, who made a careful cytological study of the bone marrow, shows a set of drawings made from serial sections of the same megalocaryocyte, where one can see the diversity of aspect of the cytoplasm and nucleus, and may receive the impression that there are various types of cells. Nevertheless, in the saponin-injected rabbits we have seen megalocaryocytes strikingly different in structure. These we may describe before separating them into groups.

These cells vary in size. The smallest are slightly larger than circulating monocytes, while the largest measure about 200μ in diameter. Generally, the cells are round or oval, but they may be reniform, crescent-shaped, fusiform or almost triangular in outline. Some cells show pseudopod-like processes of varying size, form and number. These amœboid changes can be very well observed in sections of the liver where the megalocaryocytes inside the capillaries are greatly elongated and cylindrical in order to permit their passing through. The outline of the megalocaryocytes is either sharp, coarse or diffuse; sometimes there is a bay-like depression.

Many authors have described the structure of the cytoplasm of the megalocaryocytes and, since the classical work of Wright on blood-platelet formation, this question has been thoroughly and carefully studied. Our findings are closely similar to what has been described. Indeed, there are cells in which the cytoplasm is apparently homogeneous and basophilic. Frequently the cytoplasm of these cells shows one or more homogeneous eosinophilic areas of different size and shape, which may have been produced by the fusion of several phagocytized erythrocytes. Some cells have a more abundant cytoplasm, apparently homogeneous, but less basophilic, often with the same oxyphilic areas above described; while in others the protoplasm is slightly acidophilic. Many cells, however, with a basophilic or slightly eosinophilic cytoplasm, show a granulated structure with the differentiation of zones as described by Wright, Foa, Schridde, Cesaris, Demel, Di Guglielmo, Ferrata and many others. These cells show a cytoplasm generally formed by two zones, usually sharply distinct: the large, granulated,

perinuclear zone and the narrow, apparently homogeneous, peripheral zone, showing a delicate basophilic or a light acidophilic reaction. The granules, very small, round or elongated, are stained lilac or violet, and are generally arranged in more or less definite parallel rows, circumscribing the nucleus. Some cells show an irregular granulation, with small and large granules, the latter generally lying near the periphery of the cell. As these large granules are separated one from another and embedded in a basophilic protoplasm, each granule is surrounded by a delicate zone, bluish in color. The cytoplasm of the megalocaryocytes frequently shows its phagocytic activity by containing several embedded cells; sometimes there are cells lying in small depressions of the cytoplasm, showing the act of engulfing.

The nuclei also present a great variety of shape and size, often due to projection of their sectioned portion, as mentioned above. According to their strikingly different structure, it is easy to distinguish several types:

1. Large nuclei, very complicated in form, unevenly globular and hollow, with some irregular openings that permit the communication of the cytoplasm, situated inside and outside. These nuclei have been compared to a "basket-work," composed of threads twisted in the most complex way. They vary greatly in aspect, according to the level through which the section passes. The sections near the periphery show the nucleus as formed by more or less numerous small pieces grouped together, each one seeming to be limited by a definite membrane. The sections near the centre, through the equator, for instance, show a ring or wreath-shaped nucleus, formed by many nuclear pieces, irregularly joined by their extremities.

The chromatin is dispersed in more or less numerous granules of various size, probably supported by a reticulum of *linin*. These nuclei are full of nuclear juice, or *paralinin*, so that they appear as though formed by deeply stained basophilic granules embedded in a colorless or slightly basophilic substance.

2. Small round nucleus of the same fine structure.

3. Large or small nuclei of various and very irregular forms, such as to defy any description. These nuclei are so rich in chromatin that the entire nuclear body is homogeneously very deeply stained, almost black, giving the idea of irregularly spread ink spots. These are very pyknotic nuclei.

4. A nucleus that appears composed of many short segments of a very dense, deeply stained chromatin, giving the impression of numerous *chromosomes*. Sometimes the thread of dense chromatin seems to be continuous, like a *loose spireme*—*luto spireme*. The nucleoli in these types of nuclei are sometimes very conspicuous and acidophilic.

These different conditions of the cytoplasm and nucleus of the megalocaryocytes perhaps are the result of different evolutionary phases of the same kind of cell.

In resuming, we have seen the following types of cells or probably the various successive evolutionary phases of the same type of megalocaryocyte:

1. Small cells with a deeply stained basophilic cytoplasm, apparently homogeneous, and a small round, oval or reniform nucleus, rich in nuclear juice (megalocaryoblast of Ferrata ?).

2. Large cells with an apparently homogeneous and either basophilic (lymphoid megalocaryocyte of Ferrata) or acidophilic cytoplasm, and a complex "basket-work" nucleus, rich in paralinin.

3. Large cells with a granulated cytoplasm and a complex "basket-work" nucleus, rich in nuclear juice (granulated megalocaryocyte of Ferrata).

4. Cells of various sizes with an apparently homogeneous protoplasm and an irregular pyknotic nucleus (degenerate megalocaryocyte of Carnegie Dickson ?).

5. Cells of various sizes, the nucleus of which has the appearance of a mitotic figure—*luto spireme*—or chromosomes in formation (megalocaryocyte with nucleus in act of dividing or of rearrangement?).

6. Free nuclei, apparently without protoplasm at all. These nuclei are frequently pyknotic but sometimes vesicular.

Our cytological studies have been made with paraffin sections cut at 4.5μ and stained with Wright's stain.

That the megalocaryocytic reaction is a specific reaction to the most urgent needs of the blood, was shown by our Rabbit No. 11. This rabbit, which had been splenectomized, after having received many injections of saponin had reached a state of equilibrium between the destructive and repairing processes of the blood. When killed, the bone-marrow of this animal presented the usual lesions which impair partially its functional capacity; there was a slight myeloid metaplasia in the liver; but—and this is the interesting point—there was a megalocaryocytic reaction in almost all the lymph nodes studied.

We had never found megalocaryocytes in the lymph nodes of our other rabbits (Rabbits 21, 22, 23, 24, 25). What happened in this case would be explained by those who admit the unicist theory of the secondary blood formation, in the following manner: In the lymph nodes there are cells which cannot be considered morphologically different from the haemocytoblast (Ferrata) or lymphoidocyte (Pappenheim) of the bone marrow, as has been admitted by Dominici, Maximow, Pappenheim, Ferrata, and others. Usually in the lymph nodes these cells give rise to lymphoid elements and are, as Ferrata clearly expresses it, haemocytoblasts in lymphoid function; but, like the medullary haemocytoblasts which produce megalocaryocytes and other myeloid cells in the bone marrow, in special conditions the haemocytoblasts of the lymph nodes can produce these megalocaryocytes or other myeloid elements. The myeloid potentiality of the lymph-

nodes has been stimulated, in the case of Rabbit 11 by the absence of the spleen. That the myeloid potentiality of the lymph nodes has been specifically stimulated to form almost only megalocaryocytes, seems to show that the need of these cells or of the elements deriving from them, was the greatest.

MECHANISM OF THE DISTURBANCE IN THE BONE MARROW

During the first hours following an injection of saponin, we noticed an extreme dilatation of all the capillaries of the bone marrow, which are packed with blood. This occurs, however, when other blood poisons are injected into rabbits, as, for example, benzol. We confirm this observation on the effect of benzol injected intravenously, on two rabbits (No. 14 and No. 16). After the intravenous injection of saponin, however, this hyperaemia is almost immediately followed by hæmorrhages due to rupture of the overfilled capillary. This does not occur, so far as we know, in benzol poisoning. The question arises whether the rupture of the capillaries may not be due to an injury and weakening of the capillary wall.

It is, however, not easy to understand why this action of saponin is localized only in the capillaries of the bone marrow, since we do not find any hæmorrhages in other organs. Whatever the cause of these hæmorrhages, it may be that all the other changes are consequences or a response to them. Necroses of some portion of the bone marrow are easily understood as a consequence of an impairment of its normal blood-circulation.

Organization of the necrotic foci or the foci of hæmorrhages is also a natural reaction. The general result of this process will be a partial incapacity of the bone marrow, which, in the areas in which the normal circulation is maintained, shows a high degree of blood formation. This is certainly different from a complete aplasia of bone marrow, such as saponin is classically said to produce.

Returning to the history of the case which determined our experimental study of anæmia following saponin poisoning, we are forced to admit that although saponin gives a clinical picture and autopsy findings in rabbits comparable to those given by the above described patient, there are very important differences which have to be emphasized. Like the spleen and liver of the patient, these organs in our rabbits showed an intense megalocaryocytic reaction; like his blood, their blood contained few red cells, numerous normoblasts, very few myelocytes in rare cases, and very few platelets. There are, however, striking differences: whereas the patient had a low color index and a fatty bone marrow, the experimental animals always had a high color index. There was a high grade of hæmoglobinæmia and hæmoglobinuria (as, for instance, Rabbit No. 4). All our rabbits at autopsy had a hyperplastic bone-marrow, although with its normal capacity very much diminished by diffuse hæmorrhages. The pathological problem of the case described concerns the one-

sided blood-formation, but cannot be explained by our findings with saponin poisoning.

We have to admit that we were dealing in this case with another etiological factor and another mechanism. If any suggestion might be made, we would say this: it is improbable that any toxic factor in the body would prevent the formation of myeloid tissue where it normally exists, while at the same time it would allow the building up of myeloid tissue in organs where it is not found in the normal condition. Whatever the etiological factor in such a case may be, it is probable that it acts indirectly on the myeloid tissue of the bone marrow. Perhaps a careful study of the vessels and the nerves of the bone marrow, the functions of which are not well known and have not been thoroughly studied, would be of a greater help.

CONCLUSIONS

1. Single or repeated intravenous injections of saponin in rabbits do not change the resistance of the red blood cells to this toxic substance.
2. The erythrocytes of splenectomized rabbits are more resistant to hypotonic salt solution but not to saponin.
3. In addition to its hæmolytic action saponin is a highly destructive agent for blood platelets, both *in vivo* and *in vitro*.
4. The intravenous injection of saponin into rabbits produces in the bone marrow hyperplasia and, simultaneously, numerous diffuse or circumscribed foci of hæmorrhage; there is not a true aplasia.
5. As the function of the bone marrow is impaired by hæmorrhages and their effects, the spleen, liver and other organs show a vicarious myeloid formation. This newly grown myeloid tissue shows an unusually large number of nucleated red blood cells but principally megalocaryocytes—megalocaryocytic reaction.
6. A megalocaryocytic reaction was observed in the lymph glands, after intravenous injection of saponin, in splenectomized but not in non-splenectomized rabbits. This may be explained as a vicarious function.
7. The megalocaryocytes found in the bone marrow, spleen, liver and other organs of the rabbits injected with saponin are not all identical in appearance. While presenting differences in morphology and structure of the nuclei and cytoplasm, they may be different stages of the same type of cell.
8. For a good understanding of the pathological changes in the bone marrow, it is very important to study large longitudinal sections (of almost the whole organ in rabbits) and cross-sections of the marrow of several bones. This is absolutely necessary in the study of the bone marrow of saponin-injected rabbits.

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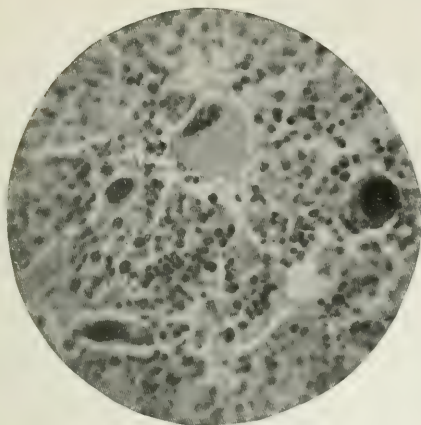


FIG. No. 1

Spleen of rabbit No. 3.

Myeloid reaction with numerous megakaryocytes.



FIG. No. 2

Liver of rabbit No. 3.

Myeloid reaction with numerous megakaryocytes.

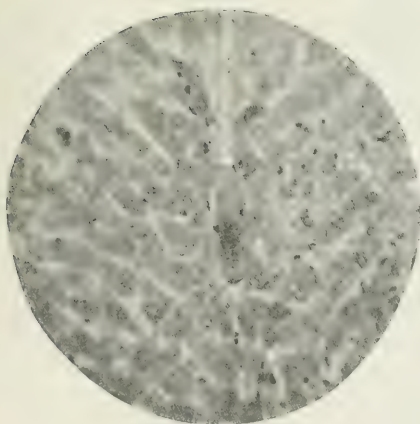


FIG. No. 3

Liver of rabbit No. 3

showing a megakaryocyte in a capillary

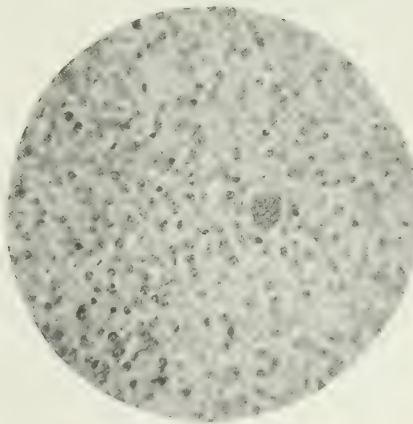


FIG. No. 4

Adrenal of rabbit No. 3

showing a megakaryocyte in the cortical tissue.

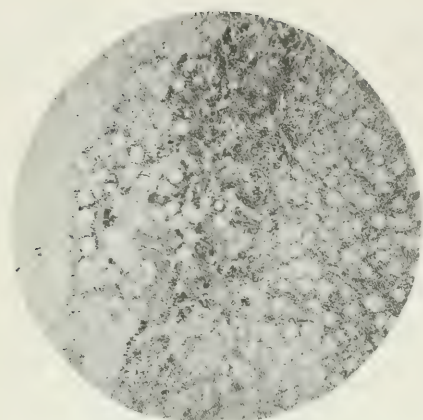


FIG. No. 5

Bone marrow of rabbit No. 12.

Diffuse hæmorrhage and vicarious hyperplasia.

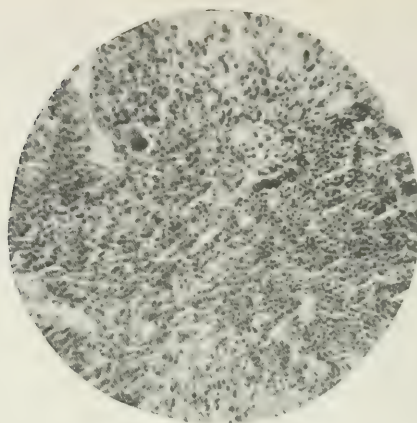


FIG. No. 6

Lymph gland of rabbit No. 11.

Myeloid reaction; a megalokaryocyte is seen in the lymph sinus.



FIG. No. 7

Bone marrow of rabbit No. 22

showing hyperplasia and a large zone of hæmorrhage.

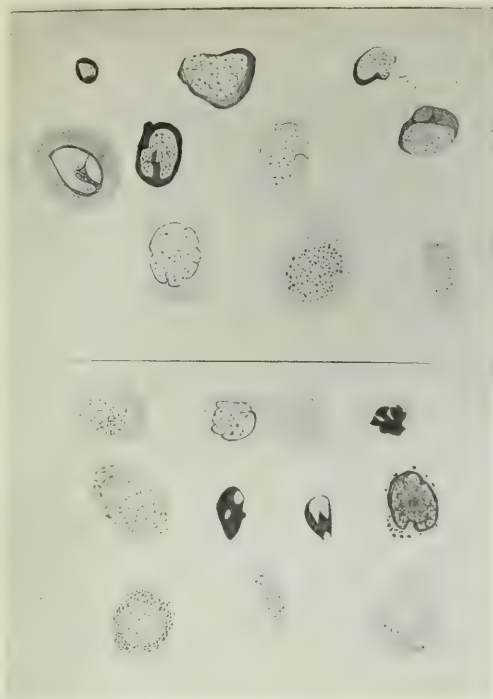


FIG. No. 8

Megalokaryocytes from the spleen of rabbit No. 3 (above) and of rabbit No. 11 (below).

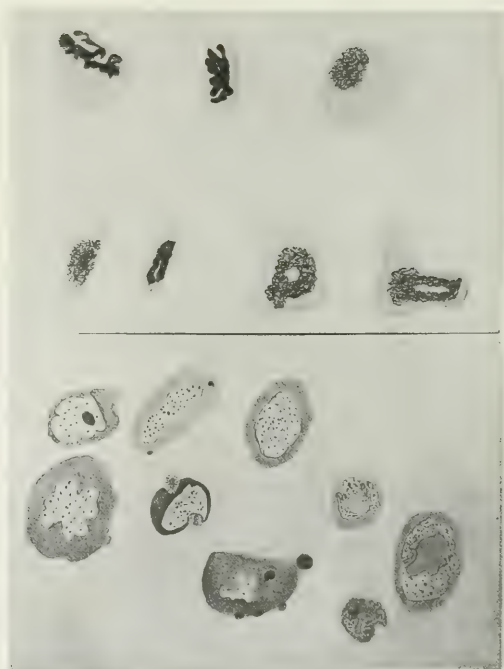


FIG. No. 9

Megalokaryocytes from the spleen (above) and liver (below) of rabbits No. 5 and No. 3.

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THE SURVIVAL OF CELLS AFTER THE DEATH OF THE ORGANISM

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INTRODUCTION

It is now a matter of common knowledge that cells of certain types can survive for a limited period after removal from the body. The ectoderm, especially noteworthy in this regard, appears to be able to live several days if kept under proper conditions. The test of viability is the "take" of the skin graft. Skeletal, heart, and smooth-muscle fibers can be made to contract and relax for a considerable period of time outside the body. The muscle-fibers in the physiological preparations used for such experiments are supposed to remain alive; the criterion here is the ability of the muscle to contract and relax in a proper artificial environment with suitable stimuli. In muscle-nerve specimens the isolated nerve-fibers are able to conduct stimuli to the muscles for varying periods. Do the axones continue to live, after their separation from the cell bodies, as long as the nerves are able to conduct stimuli to the muscles?

Carrel ('07) succeeded in getting good results from the hetero-transplantation of blood-vessels that had been kept in cold storage for 4 days, while those kept 7, 17, and 20 days, respectively, were not so successfully transplanted. It is not clear from his data whether the cells in the transplanted 4-day blood-vessel remained alive or whether a process of substitution made the transplant a success. In 1910 he succeeded in replanting spleen 44 minutes, and kidney 50 minutes, after removal from the body. The criterion for life in such experiments is the success of the transplantation and the resumption of function of the organ.

Tissue-cultures have shown that most cells not only survive but move about and even multiply for many days after removal from the body. Fischer ('21) cultivated epithelial cells for over two months, while the connective-tissue cells may live and multiply for years (Ebeling, '21). It is probable, therefore, that many types of cells are potentially immortal, given the proper environment. Tissue-culture affords the most advantageous environment that has yet been devised.

In the present study we have endeavored to determine the periods of survival of several different types of cells after the death of the animal, under various conditions other than those of tissue-culture. Our criterion for life was the presence within the cells of certain granules and vacuoles that have a great affinity for neutral red. The value of neutral red as a testing agent was suggested by

the following considerations. Experience with tissue-culture has shown that all types of living cells either have or can develop granules and vacuoles with a marked affinity for neutral red. This dye does not become concentrated within the living cells unless there is some pre-existing body or substance to which it can become attached. In the ordinary strengths used it does not stain the cytoplasm or nucleus, nor does it seem to injure the cells. It is a well-known fact that in dead cells of tissue-cultures the granules and vacuoles do not take up neutral red as they do in living cells, although the entire dead cell may stain diffusely if the dye is strong enough. Dead cells, but not living ones, are stained diffusely with various other dyes, such as toluidin blue (Gross, '11; Steckelmacher, '14), and benzidine dyes (Evans and Schulemann, '14), Nile blue B extra and brilliant cresyl blue (Lewis and Lewis, '15).

Granules and vacuoles that have been colored with neutral red in the living cell lose their color when the cell dies (W. H. Lewis, '21). We have noted repeatedly that when the cells of a spread are killed rapidly by heating, the red vacuoles lose their color and the cells become more or less diffusely pink. Much the same results were obtained when the spreads were killed with acetic acid.

As a rule, the dead cells in the various spreads, except those from the intestine, retained their form both as to cytoplasm and nucleus. It was possible to distinguish the dead from the living cells even without the use of neutral red. The main differences are in the texture of the cytoplasm and nucleus and in the character of the nuclear membrane, which becomes more marked after death.

Most cells, under abnormal conditions, develop within their cytoplasm these granules and vacuoles that have an affinity for neutral red. The process takes place not only under the usual conditions of tissue-culture, but even more rapidly under special conditions, such as the introduction of typhoid bacilli into the medium (M. R. Lewis, '20) or the exclusion of dextrose (M. R. Lewis, '21). Similar granules and vacuoles accumulate rapidly in connective-tissue cells and blood-cells, which normally possess few if any, when their supply of oxygen is cut off by sealing the spread under a coverglass (Prigosen, '21). They are also produced in the cells of living animals under the action of numerous acid-azo dyestuffs (Evans

and Scott, '21). We have found that these neutral-red bodies also accumulate in various types of cells that remain alive in the dead animal, or in organs or parts of organs that have been removed, even when they do not appear to be present in the normal, healthy cell. At the death of the animal all the cells of the body are subject to great functional disturbances of one kind or another. With the cessation of heart action all the tissues are immediately deprived of their chief source of oxygen, their food supply, and the usual pathway for the elimination of waste products. Lack of oxygen and food and the accumulation of waste products are probably all concerned in the production of the granules and vacuoles.

In these experiments most of the observations were made on the adult white rat, with a few additional ones on the guinea-pig, rabbit, and man. There were four series of experiments, with numerous repetitions of the individual observations. The rats were killed with ether, chloroform, or a knock on the head. The method of killing caused no variation in the subsequent formation of granules and vacuoles in the cells.

SERIES I. Fourteen rats were used in this series. Various organs were cut out aseptically immediately after death, put into a moist chamber (sterile petri dishes) and kept at 37° F. in an automatically regulated refrigerator for varying periods of time (24, 36, 48, 60, 72, 96, 120, 144, 168, 192, and 240 hours). Small pieces of the organs were then cut up at room temperature in about 5 c.c. of Locke's solution (NaCl .9, CaCl .024, KCl 0.42) without the sodium bicarbonate, plus 1 or 2 drops of a 0.5 per cent solution of neutral red in distilled water. After allowing a few minutes for the dye to penetrate, spreads were examined during the next few hours.

SERIES II (22 rats). Small pieces of tissue were taken from the various organs immediately after death, cut up into minute fragments in the neutral-red solution mentioned above and kept at room temperature (about 70° F.) in the small petri dishes in which they were cut up. In some of the experiments the tissues were handled aseptically. Spreads were examined every few minutes during the first hour or two, then at about one-hour intervals during the remainder of the first 24 hours. The intervals were then lengthened and spreads were examined at about 30, 36, 48, 60, 72, 96, 120, and 144 hours after death.

SERIES III (14 rats). The entire animal was kept at 37° F. for varying periods—6, 12, 24, 36, 48, 72, 96, and 120 hours. Small pieces were then cut up in the neutral-red solution and examined as spreads.

SERIES IV (8 rats). The entire animal was kept at a temperature of about 70° F. for varying periods (1, 2, 6, 9, 12, 18, 24, and 48 hours) after death. Small pieces were then removed and cut up for spreads in the neutral-red solution in the usual manner.

After allowing a few minutes for the dye to diffuse into the minute pieces in each of the above series, the fragments were more or less flattened out under a coverslip in a drop of the fluid, thus forming the spreads for examination. Such spreads can be examined with the oil-immersion; when the granules are first beginning to form, high powers are necessary to recognize them, since they are exceedingly minute. Later, as the granules and vacuoles become larger, they can be recognized with the dry lenses or even with the high power Greenough binocular. The latter type of microscope is very useful for examination of entire organs and large pieces in the dishes. The mechanical injury with resulting death of the cells, produced by cutting the organs with scissors, extends to varying depths in the different organs and tissues composing them, so that along the cut edge there is often a diffuse pink stain. The neutral red does not penetrate very rapidly into the larger pieces; thus the surface may be brilliantly stained while the cells just beneath show scarcely any trace of color. The epithelial lining of the bladder, trachea, and uterus, and the surface of the liver and kidney are very beautiful when the cells become loaded with red vacuoles.

OBSERVATIONS ON RATS

In the following table are given the maximum intervals after the death of the animal in which red granules and vacuoles were observed in the various types of cells. These figures approach closely the extreme limit of survival under these several conditions. It will be seen that cells survived longest when the organs were cut out and kept at 37° F. (Series I). The cells of tissues cut up into minute fragments and kept at room temperature in Locke's solution without sodium bicarbonate (Series II) survived about as long as when the entire animal was kept at 37° F. (Series III). When the entire animal was kept at room temperature the cells did not survive nearly as long as those in the other three series. It must be borne in mind that there were, as a rule, but few surviving cells in any of the spreads at the extreme time limit and often spreads were observed in which none of the cells contained red vacuoles. It is more than likely that renewed observation and more diligent search would reveal a slightly longer life for most of the cell types mentioned in the table and might considerably increase that for some of the others, as for example, the endothelium in series III.

The table also shows under series II the earliest period at which red granules and vacuoles were observed in the various types of cells. The observations are not complete, but they serve to show that most types of cells begin very quickly after death of the animal to develop granules and vacuoles that have an affinity for neutral red. In smooth muscle, endothelium, and red blood-corpuscles there was sometimes a considerable delay before

any red granules or vacuoles appeared. In the macrophages they were seen at once and appeared very rapidly in most of the other types of cells.

In series I, III and IV, when the organs or animals were kept for some time before examination, the various types of cells probably developed the granules and vacuoles before the tissues were cut up in the neutral-red solution, with the exception of the smooth muscle and endothelium of the animals kept only one or two hours in series IV. Otherwise, in these series it was found that the cells when first examined contained about the maximum number of granules and vacuoles. In series II, however, where the tissues were examined as rapidly as possible after death, there was a gradual increase in the number of red granules and vacuoles in most types of cells during the first few hours until a maximum was attained. This remained more or less stationary for varying periods, according to the type of cell, and then decreased until all color was lost from the granules and vacuoles. Near the end some spreads showed a few surviving cells among thousands of dead ones, while others of the same group contained no living cells at all. No spreads, even those earliest observed, were made up entirely of living cells. Many cells were undoubtedly killed by mechanical injury; most spreads of the various tissues showed areas in which all or most of the cells were dead and other areas in which many cells were alive, that is, contained red granules and vacuoles. There is a comparatively short period near the end when it is difficult to decide whether a cell is alive or dead, but this does not materially influence our results, as we have utilized only the more positive data from the many observations.

Large macrophages, crowded with red vacuoles, were observed from 24 to 240 hours in series I; in series II, from 5 minutes to 144 hours; in series III, from 6 to 120 hours, and in series IV, from 1 to 24 hours after death. The vacuoles, apparently normal inclusions of these cells, take up the neutral red as rapidly as it penetrates to them. They seem to have the maximum number of vacuoles from the very outset of the observations. The vacuoles do not seem to be of quite the same character as those which develop in most of the other types of cells after the death of the animal, and it is very probable that they do not indicate such a rapid degeneration or injury as do the vacuoles in other types of cells. In series I, the red vacuoles were still present at 240 hours in the macrophages of the diaphragm, tongue, lungs, bladder, trachea, and fat; in the salivary glands at 192 hours; in the heart, thymus, and uterus at 168 hours; in the spleen at 120 hours; and in the epididymis and pancreas at 96 hours. They usually survive as long or longer than most of the other types of cells in the organ. Near the end of their period of survival the number of macrophages with colored vacuoles often decreases, the color changes to a

TABLE

SERIES	I.	II.	III.	IV.
	Hrs.	Hrs.	Hrs.	Hrs.
Large macrophages.....	240	5'-144	120	24
Tracheal cartilage.....	240	20'-144	120	24
Kidney epithelium.....	240	10'-120	120	6
Smooth muscle.....	240	90'-120	12	3
Salivary gland epithelium.....	192	16'-120	96	3
Bladder epithelium.....	192	12'-120	72	3
Tracheal epithelium.....	192	10'-120	120	6
Tongue epithelium.....	192	45'- 48	96	
Endothelium.....	168	35'- 72	12	3
Small lymphocytes.....	168	25'- 48	60	6
Large lymphocytes.....	168	25'- 48	60	6
Microcytes.....	168	25'- 48		3
Lung epithelium.....	144	35'- 52	48	3
Leucocytes.....	120	45'- 24	36	1
Kupffer cells.....	120	20'- 49	60	12
Brain macrophages.....	96	10'- 48	96	12
Pancreatic epithelium.....	96	30'- 49	96	3
Red Blood-corpuscles.....	72	53'- 24	24	1
Liver cells.....	96	20'- 15	12	
Sertoli cells.....	96	7'- 12		3
Mesenchyme.....	72	17'- 72	48	3
Ovarian follicular cells.....	72	30	48	1
Uterine epithelium.....	72	20'- 53	12	1
Uterine gland cells.....	72	24	12	1
Epididymis epithelium.....	48	55'- 18		3
Adrenal cells.....	24	15'- 32		3
Fat.....	0	18		1
Intestinal epithelium.....	0	60'- 18		
Nerve cells.....	0	10'- 1		0
Skeletal muscle.....	0	0 0		0
Heart muscle.....	0	0 0		0

reddish brown, and the distinction between living and dead cells becomes uncertain. The large macrophages were very abundant in the lungs, loose mesenchyme, skeletal muscle, organ capsules, and fat (Figs. 1, 2, and 3). They were fairly numerous in the uterus, bladder, spleen, lymph nodes, thymus, and blood clots, and less so, as a rule, in the heart, ovary, and epididymis. In the kidney, pancreas, salivary glands, testis, and liver they are comparatively rare and probably occur only in the connective septa, so that they were not often seen in spreads. They were not seen in the cortex, cerebellum, cartilage, ectoderm, or adrenal. The macrophages of the cortex and cerebellum and the Kupffer cells of the liver are of a somewhat different character.

Tracheal cartilage is one of the most persistent of tissues. In series I red vacuoles were seen in its cells from 24 to 240 hours, in series II from 20 minutes to 144 hours, in series III from 6 to 120 hours, and in series IV at 1 to 24 hours after death. There was a considerable increase in the size of the vacuoles as the period after death lengthened. Macrophages were very abundant in

the peritracheal mesenchyme and in these the red granules and vacuoles usually persisted longer than those in the other cells of the trachea, with the exception of the cartilage.

The *epithelial cells of the kidney tubules* developed many granules and vacuoles in each series (Fig. 6). In series II, in which the development of these granules and vacuoles was followed, there was at first little or no red color, but during the next 3 or 4 hours red granules and vacuoles appeared, gradually increasing in number and size until the cells were crowded with them. This condition remained more or less stationary for varying periods. Red granules and vacuoles were observed in the cells from 24 to 240 hours in series I, from 10 minutes to 120 hours in series II, from 6 to 120 hours in series III, and from 1 to 6 hours in series IV. Most of the spreads showed dead cells diffusely colored, as well as cells containing red vacuoles. The number of cells containing red vacuoles gradually decreased as the period after death lengthened, until only a few could be seen, and finally the color disappeared from these also. The neutral red did not penetrate the larger pieces very rapidly, so that for a time only the uninjured cells on the surface of the larger pieces contained the red vacuoles. In many specimens every cell in the tubules on the normal, uncut, uninjured surface contained vacuoles, while only a part of those on the cut surface showed them, the injured cells being diffuse pink in color. At first only those cells on the superficial side of the tubules on the uncut surface contained red vacuoles, while the deeper ones, to which the neutral red had not penetrated, contained very little color. When, after two or three hours, fresh cuts were made across the larger pieces, the cells in the newly exposed tubules usually showed little or no red color. Vacuoles were undoubtedly present, for the uninjured cells along this surface accumulated neutral red quite rapidly, much more rapidly than when the tissue was first removed.

In the glomeruli red granules were seen, but whether they were in the epithelium, in the endothelium, or in both types of cells could not be determined with certainty. Macrophages are very rare in the kidney.

Smooth muscle was noted in the bladder and uterus and occasionally in blood-vessels. This tissue, like endothelium, does not appear to develop vacuoles as rapidly as most of the other tissues. The vacuoles have a characteristic linear arrangement (Fig. 7). In series I, red vacuoles were seen from 24 to 240 hours, in series II from 1½ to 72 hours, and in series III from 6 to 12 hours. Further observations would probably show a longer persistence of vacuoles in series III. In series IV the smooth muscle was examined at 1 and at 3 hours, but no vacuoles could be seen; later, however, spreads from the same dishes did show numerous red vacuoles

in the smooth muscle. Either the neutral red had not penetrated or the vacuoles had not developed at the time the first observations were made.

Salivary-gland epithelium became heavily loaded with red granules and vacuoles (Fig. 18). There was usually a clear peripheral zone that varied in width in different glands. It was not so pronounced as in the pancreas. The colored vacuoles were crowded in among the secretion granules, which did not take up the neutral red. The number of colored vacuoles seemed to vary in direct proportion to the number of secretion granules. Red granules and vacuoles were found from 24 to 192 hours in series I, from 16 minutes to 120 hours in series II, from 6 to 96 hours in series III, and from 1 to 3 hours in series IV. In series II the duct epithelium was occasionally observed (120 hours) to be loaded with red granules and vacuoles, while very few of the gland cells contained them, most of the latter being dead. Macrophages were not common.

The *bladder epithelium* appears to consist of two rather distinct types of cells. When first examined from 10 to 60 minutes after death in series II, the cells contained only a few small granules and vacuoles, but during the course of the next 3 or 4 hours these increased greatly in both number and size. At this time a marked difference in their reaction to neutral red becomes apparent in the two types of cells. The larger type, often binucleated or multinucleated, developed many granules and vacuoles that became very heavily laden with the neutral red (Figs. 13 and 14). These cells, less numerous but much larger than the second type, are scattered in more or less irregular patches throughout the entire epithelial lining. When viewed with the binocular dissecting microscope, they stand out very conspicuously, especially the many large giant cells. The second type, smaller in size, has fewer and less deeply staining granules, arranged in a circle around a relatively large nucleus. Since the epithelial cells are on the surface, the neutral red has easy access and probably diffuses into the cells as rapidly as the granules and vacuoles form, and the slow increase during the first 3 or 4 hours probably is a fair indication of the rate at which the vacuoles and granules develop. The larger cells seem to develop vacuoles at a faster rate than the smaller ones and to reach a maximum earlier, maintaining this maximum for a considerable period of time. In large pieces of the bladder one can see the brilliantly stained epithelial surface in marked contrast to the pale muscular layers, for only after several hours does the latter accumulate enough red vacuoles to become conspicuous. This is due partly to the slowness of penetration by the dye. When the epithelial wall of the bladder is thrown into folds, as it usually is in the larger pieces, the neutral red appears first in the high spots of the folds, often long before the cells in the depths show any appreciable color. This is probably

merely a question of diffusion of the dye in the solution itself. Red granules and vacuoles were observed in the cells from 24 to 192 hours in series I, from 12 minutes to 120 hours in series II, from 6 to 72 hours in series III, and from 1 to 3 hours in series IV. Macrophages are fairly abundant in the bladder wall and live longer than most of the other types of cells.

The *tracheal epithelium* was about as persistent as the cartilage but was not always present in the spreads. In series I, red granules and vacuoles were seen from 24 to 192 hours, in series II from 10 minutes to 120 hours, and in series III from 6 to 120 hours. In series IV the epithelium was observed at 1 and 6 hours; it probably lived longer, but unfortunately our spreads did not contain these cells at later periods. It was possible to watch with the low power the gradual accumulation of red color in the epithelial cells of large pieces, until finally the whole lining of the trachea was deep red. Ciliary movement was observed in the bronchi 120 hours after death in series I and in series III.

Observations on the *tongue ectoderm* were unsatisfactory. In series I, red granules were seen at 24, 48, and 192 hours but not at 72, 96, 120, 168, or 240 hours. In series II, red granules were seen in some of the cells at 45 minutes, 4 hours, and a very few at 48 hours, but none at 13, 18, 36, 96, 120, or 144 hours. In series III, granules were found at 48, 72, and 96 hours, but none at 12, 24, or 60 hours. In series IV, although observations were made from 1 to 24 hours after death, no red granules were seen in the cells. On the base of each papilla there developed a superficial area of pink granules or small vacuoles. These differed so markedly from the other vacuoles and granules that we have not considered them as indicators of the condition of the cells. Other superficial cells did not develop granules or vacuoles and they probably prevented the penetration of the neutral red to the deeper layers. At the edge of the cuts the cells were killed and the deeper layers of the epithelium were stained a diffuse pink. The granules and vacuoles occasionally seen in the cells of the deeper layers were observed only in spreads from exceptionally favorable pieces, and we doubt if our data give an adequate idea of the period of survival of these cells. Macrophages were very abundant in the connective-tissue spaces beneath the ectoderm and retained their red granules for 240 hours in series I, 72 hours in series II, 96 hours in series III, and 12 hours in series IV.

Endothelium was observed in the capillaries of various organs. The granules and vacuoles that take up the neutral red did not always appear to develop as rapidly as in most other types of cells. In series I, endothelial cells with red vacuoles were seen from 24 to 168 hours; in series II from 35 minutes to 72 hours; in series III, red vacuoles were seen at 12 hours; in series IV observations at 1 and 2 hours did not reveal red granules, but

the same material kept for 24 hours longer in the neutral-red solution showed cells containing red vacuoles, indicating that they were alive at the time of the first observations. The size and number of vacuoles varied greatly in different spreads and in different parts of the same spread. The endothelial cells in some capillaries were filled with red vacuoles, whereas others were entirely without them (Figs. 16 and 17). At the first observation in series II there were a very few scattered vacuoles in the capillary walls, the number slowly increasing until the cells became quite full of them.

In the thymus, *small lymphocytes*, with from one to four small red vacuoles or granules, were seen from 24 to 168 hours in series I, from 25 minutes to 48 hours in series II, at 60 hours in series III, and at 1 and 3 hours in series IV. *Large lymphocytes*, containing a greater number of red granules and vacuoles, were seen at the same periods. The *microcytes*, with one or two rather large red vacuoles, were usually seen at the same time. Large macrophages were fairly abundant and usually survived as long or longer than the other cells. Similar cells from the spleen and lymph nodes were observed. They lived longer in the thymus than in the lymph nodes and longer in the lymph nodes than in the spleen. When the lymphocytes were dying, it was not uncommon to see cells, especially the large lymphocytes, with a few large vacuoles of a brownish red color and a dead nucleus having a sharp nuclear membrane.

In the lung, *epithelial cells*, with red granules and vacuoles, were seen from 24 to 144 hours in series I, from 35 minutes to 52 hours in series II, from 6 to 48 hours in series III, and from 1 to 3 hours in series IV. In series III there were possibly a few at 60 and 96 hours but none at 72 hours. The epithelial cells do not contain many vacuoles at any time and it was not always easy to determine whether the vacuoles were in these cells or in the endothelium. The macrophages, which occurred in great numbers, were the most characteristic objects in the lungs. They were found, loaded with red granules and vacuoles (Fig. 1), in series I from 24 to 240 hours, in series II from 30 minutes to 120 hours, in series III from 6 to 96 hours, and in series IV from 1 to 24 hours after death.

Blood-cells were examined in various organs and in blood clots. Leucocytes containing numerous red granules and small vacuoles were found from 24 to 120 hours in series I, from 45 minutes to 30 hours in series II, from 6 to 36 hours in series III, and at 1 hour in series IV. Red blood-corpuscles, with one to three or four granules or small vacuoles, were seen at 72 hours in series I, from 1 to 24 hours in series II, at 6 and at 24 hours in series III, and at 1 hour in series IV. Usually, very few if any of the corpuscles examined in a spread contained red vacuoles. Large macrophages were very abundant in

blood clots that had formed in the body cavities after the organs were removed for examination.

The *Kupffer cells* were usually seen without much difficulty and when first observed contained the maximum number of red vacuoles. They were often noticed in spreads in which the liver cells were dead or without vacuoles, and hence are apparently not killed as easily by the manipulations as the latter type. The vacuoles in the Kupffer cells were somewhat different in character from those formed in the liver cells, and they also either had a greater affinity for neutral red or were more easily penetrated by it. The fact that the Kupffer cells always contained the maximum number of vacuoles at the first observation indicates that the vacuoles are normal inclusions of these cells. They were seen from 24 to 120 hours in series I, from 20 minutes to 49 hours in series II, from 6 to 60 hours in series III, and from 1 to 12 hours in series IV.

Brain macrophages were observed in both the cortex and cerebellum (Fig. 21). They were not numerous and were usually scattered along the small blood-vessels to which they sometimes seemed to cling closely. They were often elongated, especially when attached to a blood-vessel. The vacuoles did not, as a rule, stain as deeply nor were they as numerous as those in the tissue macrophages. Like those in the tissue macrophages and in the Kupffer cells, they appeared to be different in character from the vacuoles formed in the tissue cells and are probably normal inclusions of these cells, as there was practically no increase in their number. The brain macrophages are not as large as the tissue macrophages. They were observed from 24 to 96 hours in series I, from 10 minutes to 48 hours in series II, from 6 to 96 hours in series III, and from 1 to 12 hours in series IV.

The *pancreatic gland cells* developed many small red granules that were always definitely located in the lumen end of the cell among the secretion granules. At the distal end of the cell there was usually a clear zone, free from red granules and secretion granules, which varied in width in different animals according, apparently, to the state of secretory activity as already noted in the cells of the salivary gland (Figs. 8 and 9). The red granules were observed from 24 to 96 hours in series I, from 30 minutes to 49 hours in series II, from 6 to 96 hours in series III, and at 3 hours in series IV. Macrophages were very rare.

Liver cells usually developed granules and vacuoles rather slowly and the cells on the surface, especially the uncut surface, showed the red color long before the more deeply placed ones. The liver cells are apparently easily killed and in many of the spreads no living ones, or at most only small patches of them, could be seen, whereas practically all the cells on the uninjured uncut surface of the larger pieces were filled with vacuoles. The vacu-

oles varied in size and were usually scattered through the cell (Figs. 10 and 11). Occasionally, red granules were seen only in that end of the cell bordering the bile capillary (Fig. 12). The vacuoles were found from 24 to 96 hours in series I, from 20 minutes to 15 hours in series II, and from 1 to 12 hours in series III. Although numerous observations were made in series IV, no cells containing red granules or vacuoles were seen. Further tests, with due appreciation of the susceptibility of the cells to mechanical injury, would probably have revealed living cells in series IV and for longer periods in series II. The large tissue macrophages are rare in the liver.

Testis.—The *Sertoli cells* were very conspicuous objects in spreads from pieces of the testis cut up in the neutral red solution. They formed regular patterns in the walls of the tubules (Fig. 20). The long axis of these cells is normally perpendicular to the surface of the tubule, but under pressure of the coverglass they became bent parallel to the surface, especially at the cut end of the tubule (Fig. 19), where they often became detached and floated off with a group of attached spermatogonia. Red vacuoles were observed in series I from 48 to 96 hours, in series II from 7 minutes to 16 hours, and in series IV at 3 hours after death. No observations were made in series III. It seems probable that the vacuoles are normally present in these cells. Macrophages were rarely seen. Interstitial cells with red granules and vacuoles were occasionally observed as early as 45 minutes in series II. Male germ cells and moving spermatozoa with one or more red granules were occasionally seen in series II from 40 minutes to 13 hours after death.

Mesenchyme cells were observed in various places but they did not often contain red granules or vacuoles. These cells are apparently rather sensitive; the nuclei were usually stained pink, indicating that the cells were dead. We have not attempted to distinguish between the various types of cells which constitute the connective tissues, except the tissue macrophages. It is rather surprising that the mesenchyme cells did not show up better. Red granules and vacuoles were observed in the cells from 24 to 72 hours in series I, from 17 minutes to 72 hours in series II, from 6 to 48 hours in series III, and at 3 hours in series IV. These cells did not develop as many vacuoles as some of the other types.

The *follicular cells* of the ovary were rather difficult to observe in spreads and we have comparatively few observations on the granules and vacuoles. Dense tissues like the ovary and adrenal are difficult to handle by our methods. Red vacuoles and granules were observed at 24 and at 72 hours in series I, from 24 to 30 hours in series II, at 12 and at 48 hours in series III, and at 1 hour in series IV. Observations on the *ova* were very difficult and unsatisfactory. In series III many fine granules were seen in the ovum at 12 hours but none at 24 or at 48 hours. In the other series we did not suc-

ceed in getting satisfactory observations. The *germinal epithelium* contained red granules and vacuoles from 3 to 5 hours in series II. No special attempt was made to observe it in the other series.

The *uterine epithelium* developed rather small granules and vacuoles. They were first seen 20 minutes after death in series II and gradually increased in number during the next 3 or 4 hours. Large areas of the epithelium observed at this time were deep red in color under the Greenough binocular, but quite different in character from the bladder or tracheal epithelium. In series I, granules and vacuoles were observed from 24 to 72 hours, in series II from 20 minutes to 53 hours, in series III from 6 to 12 hours, and in series IV at 1 hour. The *uterine gland cells* often became loaded with red granules and vacuoles which retained their color about as long as did those in the epithelial cells.

The *epithelial cells* lining the tubules of the epididymis contained red granules and vacuoles at 48 hours in series I, from 1 to 18 hours in series II, and at 3 hours in series IV. There were no observations in series III.

The *adrenal cells* are difficult to examine by this method; few of the essential ones containing red granules could be seen, in spite of many observations. In series I, many cells appeared to contain red granules at 24 hours but none were observed from 36 to 240 hours. In series II, cells were occasionally found with red granules from 15 minutes to 32 hours, and in series IV at 1 hour. None were seen in spreads from series III. Macrophages loaded with red were found in the capsule, but none in the body of the organ.

Very rarely were *fat cells* with red granules and vacuoles observed. In series II they were found at 4 to 18 hours. None were seen in series I and III. In series IV, fat-cells examined 1 hour after death were clear, but after standing for 24 hours in the neutral-red solution red vacuoles were found in some of them. They were not seen at any other time in this series. Large macrophages were abundant (Fig. 3).

Epithelial cells from the intestine showed red granules and vacuoles from 1 to 18 hours in series II, but not from 24 to 144 hours. None were seen in series I, III, or IV. The fact that the intestine is always infected probably explains why its cells did not live long. Macrophages were not often observed.

The *nerve cells* in the cortex and cerebellum rarely gave any indication of red granules and vacuoles. A few cells with pink vacuoles and granules were seen 15 minutes to one-half hour after death in series II. Most of the nerve cells at the periphery began to show a diffuse pink at this time, indicating that they were dead. An hour later practically all the cells were a diffuse pink in color in fresh spreads. The presence of brain macrophages and endothelium with red granules and vacuoles indicated that the environment was not especially un-

friendly to life (Fig. 21). Death of nerve cells probably proceeds so rapidly that the granules and vacuoles do not have a chance to form.

Observations of *skeletal muscle* were made on the diaphragm, tongue, and abdominal muscles, but in no case were we able to find red granules or vacuoles in the fibers. Many observations were made in each series. Striated muscle behaved in a peculiar manner toward neutral red. The fibers rapidly became a diffuse deep pink, more marked than in other types of cells after death. We are unable to explain this diffuse coloring of muscle, as it is hardly to be expected that the fibers should die as rapidly as the diffuse coloration seemed to indicate. The contractile tissue evidently contains some diffuse substance that has a marked affinity for neutral red. Large macrophages, always abundant between the fibers and especially in the larger connective-tissue spaces between muscle bundles, were loaded with red granules and vacuoles.

Heart muscle behaved in a manner similar to skeletal muscle and always showed a diffuse pink. The large macrophages were not as common as in skeletal muscle, but they took up the neutral red in exactly the same manner.

A few scattered observations on the mesothelium of the body cavities, the epithelial cells of the choroid plexus (Fig. 23), the retina, iris, and cornea, showed that there is a similar accumulation of granules and vacuoles in these cells.

OBSERVATIONS ON OTHER ANIMALS

Human tissues from three autopsies were examined in the usual manner as spreads from small pieces cut up in the neutral-red solution. Tissues from the first autopsy (the patient having died of carcinoma of the stomach) were examined 6 to 7 hours after death and, although the cadaver was in the ice-box at 37°F. for an hour during this time, only a few macrophages in the lungs and capsule of a lymph node and a few Kupffer cells contained red vacuoles. The other cells from the lung, lymph-node and liver, and all the cells of the testis, kidney, spleen, and skeletal muscle that were examined, were stained a diffuse pink by the neutral-red solution in which small pieces were cut up. In the second case (a child autopsied 31 hours after death) a few macrophages containing red vacuoles were found in the diaphragm, pancreas, intestine, capsule of a lymph-node, blood clot, and thymus. The body was in the ice box at 37°F. for 15 hours of this period. None of the other tissues examined, striated muscle, epididymis epithelium, adrenal, pancreas, liver, lymphocytes, tracheal cartilage, mesenchyme, endothelium, or smooth muscle, contained red vacuoles. Some of the tissues from the third autopsy (a 7½ months' fetus) were examined about 24 hours after death. The fetus had been kept in the ice-box for about 18 hours. Lung, diaphragm, kidney, pancreas, lymph node, liver,

spleen, trachea, thymus, adrenal, and retroperitoneal tissue were examined. Macrophages with red vacuoles were found in the lymph node, spleen, thymus, retroperitoneal tissue, and capsule of the adrenal, but no other types of cells containing red vacuoles were seen.

Cells from various organs of the rabbit were examined as in series II from 15 minutes to 5 hours after death. About the same accumulation of granules and vacuoles occurred as in the cells of the rat (Fig. 15). The lungs contained many large macrophages; the adrenal cells showed a more pronounced accumulation of red vacuoles than in the rat. A fetus 6.5 cm. long, from the same rabbit, was examined especially for the macrophages in the lungs, but none were found, although these cells were numerous in the subcutaneous tissue. The epithelium of the intestine and kidney contained many red vacuoles 5 hours after death.

Cells from various tissues of a guinea-pig were examined, as in series II, from 45 minutes to 30 hours after death. The accumulation of red vacuoles and granules presented very much the same picture as seen in the rat under similar conditions (Figs. 22 and 24). The macrophages were numerous in the lungs.

DISCUSSION

Other methods might perhaps be employed which would preserve the life cells for longer periods, but these observations establish the fact that the different types of cells survive for different periods of time after death, and that the manner in which the tissues are treated makes a very great difference in the length of survival of all types of cells.

It is desirable to consider the nature of the granules and vacuoles and their probable origin before we attempt to explain why cells lived longer under some conditions than under others, and why certain cells always lived longer than others under the same conditions. With the exception of the macrophages, Kupffer cells, Sertoli cells, and leucocytes, probably none of the cells examined contained, under normal conditions in the body, more than a few, if any, granules and vacuoles having an affinity for neutral red. Such granules and vacuoles developed at different rates in the various types of cells, but we have not followed this particular phase with sufficient care to present here any definite data. Endothelium and smooth muscle often seemed to develop the vacuoles more slowly than most other types of cells. The number and size of the granules and vacuoles sooner or later attained a maximum state, which was different for each type and which was maintained for varying periods; then, as the cells approached the last phases of life, there was a diminution in the number of the red granules and vacuoles, until finally all the red color was lost. The color sometimes did not disappear from all the vacuoles until after the nuclear changes indicating the death of the nucleus

had taken place. Increased refractivity and visibility, accompanied by the appearance of a marked nuclear membrane, were the most characteristic death changes in the nucleus. Dead nuclei were easily distinguishable from living ones and were very similar to those seen in cells after fixation, before dehydration and staining. The red color that sometimes lingered in the granules and vacuoles after nuclear death indicated that not all parts of the cell died at the same time; that the nucleus might have died before the cytoplasm and that some regions of the cytoplasm died before others.

The factors involved in the production of these granules and vacuoles is not altogether clear. There is, of course, first of all, the question whether they are to be regarded as food vacuoles, as an accumulation of waste products, or as autolytic products. It is possible, though not probable, that the condition of functional stress of the cells after the death of the animal might produce such alterations in the surface layer and in the body of the cytoplasm as would permit the rapid diffusion of dissolved food and other substances from the fluids still bathing the cells and their segregation as granules and vacuoles; to these something must be added from the cell itself to give to the resulting granules and vacuoles an affinity for neutral red. Such an explanation, however, does not seem very tenable. As the cells remained alive, they must have been undergoing metabolic processes, utilizing their own substance or substances in the fluid surrounding them; in either case the difficulty of eliminating waste products would seem to be obvious and so the granules and vacuoles might be looked upon as accumulated waste products. It is very doubtful if food substances surrounding the cells are present in sufficient quantity to play any rôle, in view of the greatly reduced supply of oxygen. So for the present, at least, it seems more reasonable to consider the granules and vacuoles as the waste products of autolytic or self-digestive processes and their affinity for neutral red as a coincidence. We can hardly regard them as a segregation apparatus, as Evans and Scott (1921) have interpreted somewhat similar vacuoles found in cells after the injection of acid-azo dyes into living animals; they are much more like the degeneration granules and vacuoles described by W. H. Lewis (1919) in the cells of tissue-cultures. If, then, they are to be considered, for the present at least, as the accumulated products of autolysis, *starvation* granules and vacuoles would be perhaps as appropriate a term as *degeneration* granules and vacuoles.

Mrs. M. R. Lewis has recently shown that a trace of ammonia added to the medium of tissue cultures produces a prompt formation of vacuoles which disappear if the cultures are subsequently washed with a fresh nutrient medium. This would appear to indicate that the vacuoles are produced by the accumulation of the

waste products of nitrogenous metabolism; in other words, that they are a sign of degeneration or autolysis produced by the breaking down of the cytoplasm and an accumulation of the resulting waste products.

Wells ('06) examined histologically from time to time various tissues undergoing autolysis, noting the relative rates of alteration, especially in the nucleus. The autolytic rate in such supposedly dead tissues did not correspond to the periods of survival as determined in our experiments. Brain cells, which we found to die rapidly, according to Wells retain their essential morphological character longer than most of the body cells.

In series IV, where the entire animal was kept at room temperature, the factors contributing to the rapid death of the cells would seem to be the temperature, the spread of infection from the alimentary canal, the absence of a pathway for the elimination of waste products, and the lack of oxygen and food. The first two factors would differentiate it from series III, where the entire animal was kept on ice at 37°F. There was a very great difference in the length of survival of the cells of the two series, as can be seen readily in the table. Temperature alone is not so directly important in producing rapid death, since in series II the cells in the small fragments which were kept at room temperature lived as long on the average as when the whole rat was kept in the ice-box at 37°F. (series III). Infection may play a very important rôle, for the intestinal cells rarely lived more than a few hours in any of the series and often no living cells with vacuoles were seen at any time.

In series II (small fragments of tissue kept at room temperature in the neutral-red solution) and III (the entire animal kept at 37°F.) the survival period was approximately the same. The retardation of metabolic processes in series III was counterbalanced by more rapid metabolic processes with increased opportunity for the elimination of waste by diffusion. Infection was not entirely eliminated in series II, as only part of the tests were made under strictly aseptic conditions.

In series I infection was practically eliminated except for a few of the organs. Metabolism was retarded by the cold and the few cells that survived beyond the period for series II and III were probably favorably located.

We are hardly in a position to go farther into an explanation of the causes which bring about cell death under the conditions of our experiments. They are undoubtedly closely related to the factors which produce the granules and vacuoles. Most of the cells were probably going down hill from the hour the animal was killed.

The various types of cells must differ from each other in many ways, in chemical and physical constitution, in the rate of metabolism, in the amount of oxygen, various salts, and foodstuffs used, and in the chemical composi-

tion of the waste products. Each cell is a complex mechanism which acts effectively only in the normal environment in the animal and this environment varies more or less for each type. When the animal dies the environment is changed, whether the cells are left in the body or the tissues are removed and placed in the neutral-red solution. The period of survival in the changed environment depends on how well each type of cell is naturally fitted to live there. Now, when we consider the natural environment together with the known function of each type, we can perhaps see how this corresponds in a way with the varying periods of survival.

The tissue macrophages may owe their ability to survive for relatively long periods to the fact that they are constructed to live, under normal conditions, in the tissue spaces without close contact to either oxygen supply or special elimination paths, and that they normally contain vacuoles which are probably, in part at least, composed of waste products.

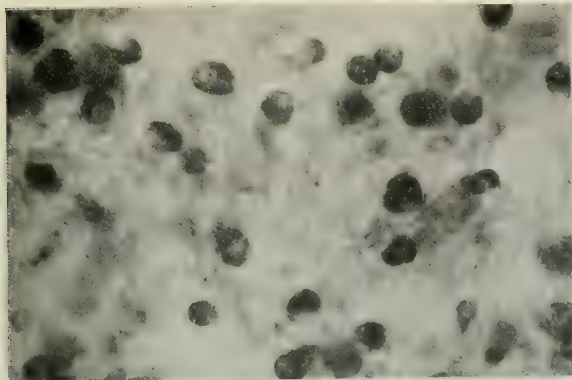
The cartilage cells likewise, though fixed in position, are naturally rather far removed from the capillaries, so that neither of these types of cells would respond to the cutting off of the blood supply as quickly as cells that have perhaps a higher rate of metabolism. Although the kidney cells are accustomed to a rich blood supply, they are also constructed to deal with waste products, so that the accumulation of waste products in the form of granules and vacuoles within them is perhaps not so detrimental as to other types of cells.

The epithelial cells from the bladder, tongue, and trachea might also be expected to survive for a relatively long period, since their peculiar location on the surfaces would indicate that they were adapted for living some distance from the blood supply.

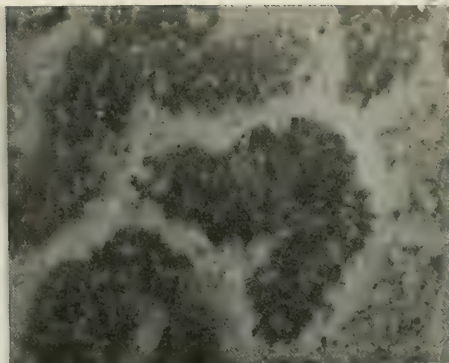
The list of cells given in the table might thus be followed in the order of their survival and reasons suggested for their relative length of survival, without, however, bringing us much nearer to the actual reasons, which are still obscure. Since in each series the environment is approximately the same for all cells, the differences in length of survival must depend on the make-up of the cells themselves.

SUMMARY

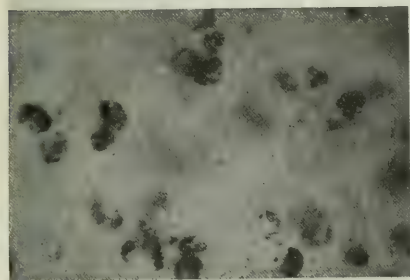
Small pieces of tissue from various organs of the rat, examined at varying periods after death, showed that most types of cells survived for a time and certain types of cells survived longer than others, in approximately the following order: tissue macrophages, cartilage cells, kidney-tubule cells, smooth-muscle cells, salivary-gland cells, bladder epithelium, tracheal epithelium, tongue epithelium, endothelium, lymphocytes, large lymphocytes, micro-



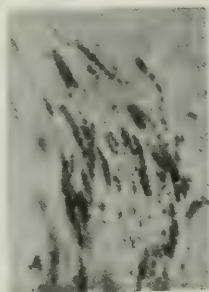
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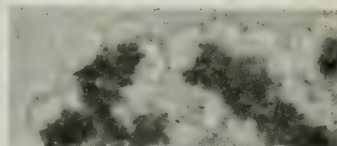
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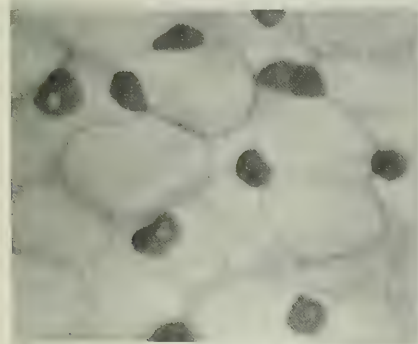
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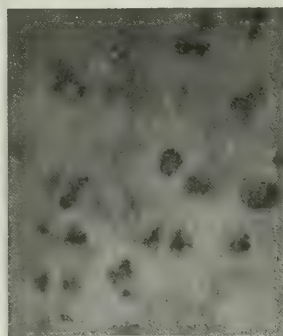
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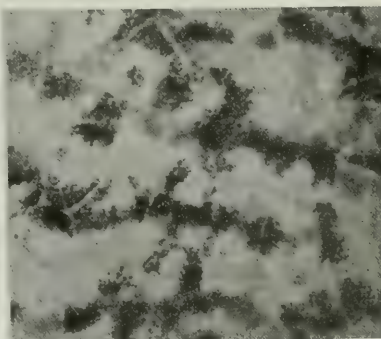
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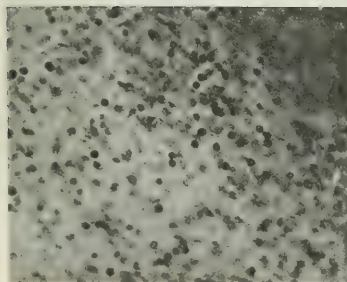
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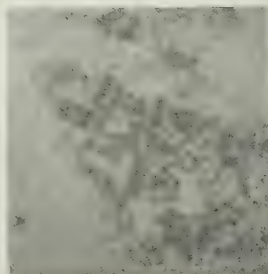
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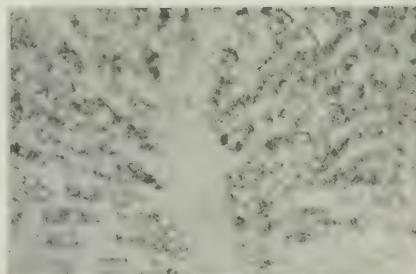
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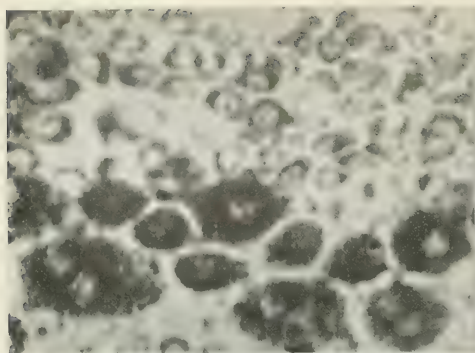
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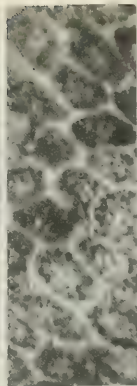
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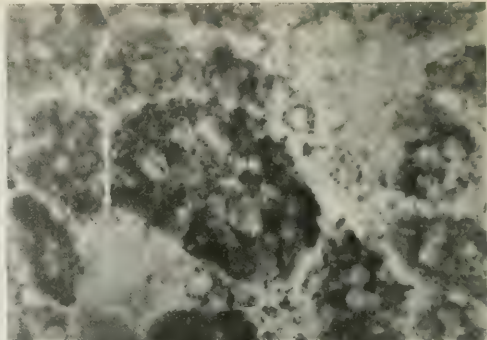
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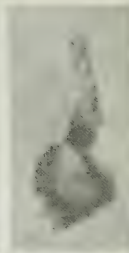
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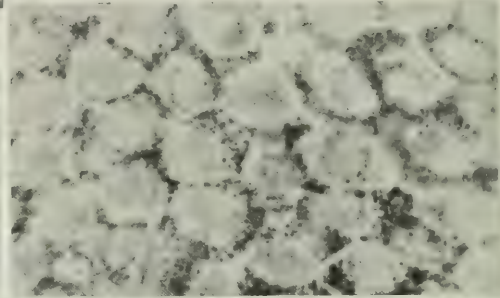
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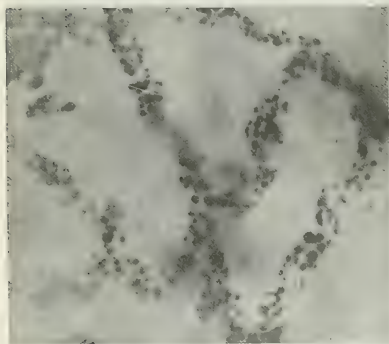
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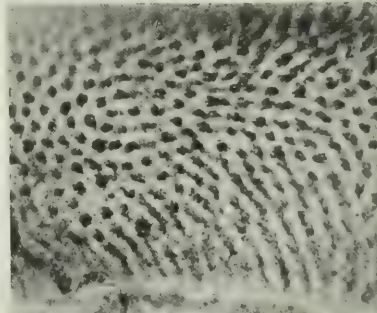
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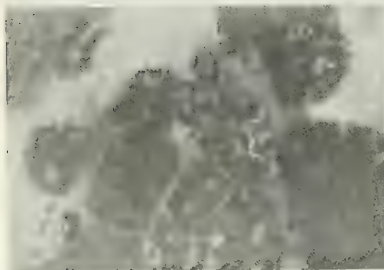
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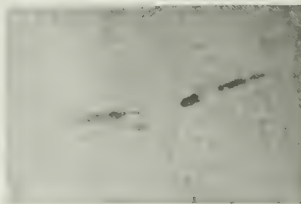
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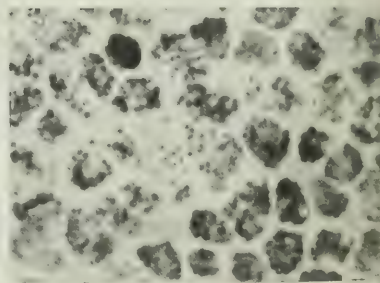
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cytes, lung epithelium, leucocytes, Kupffer cells, brain macrophages, pancreas cells, red blood-corpuscles, liver cells, Sertoli cells, mesenchyme, ovarian follicular cells, epididymis epithelium, adrenal cells, intestinal epithelium, and nerve cells. Macrophages survived for about 240 hours; brain cells for less than an hour; striated muscle developed no granules or vacuoles.

The criteria for life were: (1) the presence in cells of granules and vacuoles that had an affinity for neutral red while the cells were alive; almost all types of cells either had such granules or developed them after the death of the animal; (2) a homogeneous nucleus without trace of a nuclear membrane. The criteria for death were: (1) loss of color from the granules and vacuoles; (2) diffuse pink staining of the cytoplasm and nucleus; (3) the appearance of a sharp and distinct nuclear membrane and a change in the texture of the cytoplasm and nucleus.

Four series of experiments were conducted: In series I organs were kept at 37°F.; in series II tissues were cut up into minute fragments in a neutral-red solution and kept at room temperature; in series III the entire animal was kept at 37°F.; and in series IV the dead rat was kept at room temperature. Cells lived longest in series I, about half as long in series II and III, and a very much shorter time in series IV.

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LEGENDS FOR FIGURES

FIG. 1.—Macrophages in the lung heavily laden with neutral red granules and vacuoles. Series II, 5 hours after death. X 480.

FIG. 2.—Macrophages in the lung; much of the red color has gone. Series I, 192 hours after death. X 480.

FIG. 3.—Macrophages in fat. Series I, 36 hours after death. X 480.

FIG. 4.—Macrophages in renal capsule. Series II, 51 hours after death. X 146.

FIG. 5.—Tracheal cartilage. Granules and vacuoles near cell nuclei. Series I, 72 hours after death. X 480.

FIG. 6.—Kidney tubules. Most of the tubule cells have large irregular vacuoles. Series II, 6 hours after death. X 480.

FIG. 7.—Smooth muscle from bladder. Note linear arrangement of vacuoles. Series III, 37 hours after death; ice box 12 hours, room temperature 25 hours. X 480.

FIG. 8.—Pancreatic gland. Series I, 5 hours after death. X 480.

FIG. 9.—Pancreas. Series III; ice box 37°F. 12 hours after death. X 480.

FIG. 10.—Liver cells. Series I, 72 hours after death. X 146.

FIG. 11.—Liver cells with vacuoles. Series I, 72 hours after death. X 480.

FIG. 12.—Liver cells. Series II, 5 hours after death. X 480.

FIG. 13.—Bladder epithelium, small mononuclear, binucleate and giant cells with vacuoles. Series III, 37 hours after death; ice box 12 hours and room temperature 25 hours. X 480.

FIG. 14.—Bladder epithelial giant cells. Series II, 4 hours after death. X 480.

FIG. 15.—Bladder epithelium from rabbit, examined as in series II, 5½ hours after death. X 480.

FIG. 16.—Capillary net from ovary, vacuoles in endothelial cells. Series I, 36 hours after death. X 480.

FIG. 17.—Capillary net from bladder, endothelial cells with large vacuoles. Series II, 48 hours after death. X 480.

FIG. 18.—Salivary gland cells crowded with vacuoles. Series I, 24 hours after death. X 480.

FIG. 19.—Sertoli cells in testis tubule under pressure. Series II, 4½ hours after death. X 480.

FIG. 20.—Sertoli cells in testis tubule. Series II, 16 hours after death. X 480.

FIG. 21.—Brain macrophages. Series I, 72 hours after death. X 480.

FIG. 22.—Peritoneum from diaphragm of guinea-pig examined as in series II, 26 hours after death. X 480.

FIG. 23.—Epithelial cells of choroid plexus. Series II, 4½ hours after death. X 480.

FIG. 24.—Macrophage from lung of guinea-pig examined as in series II, 23 hours after death. X 1450.

A STUDY OF THE AUSTIN-STILLMAN-VAN SLYKE INDEX OF THE UREA EXCRETION

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and

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It is not within the scope of this short paper to attempt any review of the literature on urea excretion, and it is with considerable trepidation that one ventures to add to its bulk. Since the original work of Ambard in 1909, much evidence, clinical and experimental, has been accumulated to demonstrate that the rate of urea excretion may be used as a rough indicator of the functional capacity of the kidneys, although the mathematical formulations of the "laws" governing this rate have varied from time to time and from person to person. A detailed discussion of the subject is presented in the recent paper of Austin, Stillman, and Van Slyke, to which the reader is referred.¹ Their formulation of the relationships of the factors involved is expressed as $K = \frac{D}{BVW}$; in which D is the total urea excretion in grams for 24 hours, V is the urine volume in liters for 24 hours, B is the concentration of the blood urea in grams per liter, W is the patient's weight in kilos, and K a constant which in normal individuals has been found to vary between the limits 4.5 and 10.5. The data upon which their calculations are based were derived from repeated observations upon a few healthy subjects. It is naturally a matter of no little interest to study the behavior of the Austin-Stillman-Van Slyke constant under varying conditions of disease.

The subjects, all of whom were males, were chosen more or less at random from the public wards of the Johns Hopkins Hospital. With one exception, one observation only was made on each patient. The urine was collected usually for a period of seventy minutes; the blood for urea determination was drawn as nearly as possible at the middle of the period. There was no preceding starvation of the patients, although they were requested to abstain from taking anything by mouth for the duration of the test. In each instance, furthermore, the morning meal preceded the first voiding by at least two hours. The blood urea was determined by the Van Slyke-Cullen method. Urea of the urine was determined by the Van Slyke-Cullen modification of Marshall's urease method.² The results are presented in tabular form below.

DISCUSSION

Little comment is necessary upon Cases 1 to 9, with the exception of No. 6. Here definite urinary findings

and a decreased phenolsulphonephthalein excretion are associated with a normal index. However, the urine volume and the total urea excretion are certainly excessive, particularly as there was no polyuria, and it is reasonable to presume that the patient's slight prostatic hypertrophy interfered with the complete emptying of the bladder at the first voiding.

It is noteworthy that two cases of myocardial failure, in each of which there was evidence of renal congestion, yield indices well within the normal range. In each case, observations were made before complete recovery of compensation had occurred. In neither case was there final evidence of permanent renal damage, as judged by the usual criteria.

Case 10 was that of a patient suffering from lobar pneumonia, in cultures from whose sputa a type I pneumococcus had been recovered in large numbers. Following the therapeutic administration of 700 cc. of homologous immune serum, a well-marked urticarial rash developed, and coincidentally urinalysis demonstrated marked renal irritation. In the face of this, a normal urea excretory index was found. This result is of interest when compared with the observations of Rackemann, Longcope, and Peters on the chloride and water excretion in serum sickness.³

Two cases of pernicious anemia yielded definitely low results, while a third gave a low normal. Case 15 is of particular interest. The first estimation was made during a period of comparative quiescence, in which the red cell count had remained practically stationary for two weeks at a level just below 2,000,000, and the hemoglobin had been at 45% (Sahli). On the day of the determination, the actual count was 1,804,000, and the hemoglobin 43%. Shortly thereafter an active relapse ensued, the patient's red cell count dropping from 2,200,000 to 1,200,000 in seven days. On the fourth day of this relapse two further determinations were made, the second following the ingestion of a high nitrogen test meal, which had been given for quite another purpose. It is of great interest to note that the coefficient, which had stood at 4.33 during the remission, dropped to 3.55 during the course of the relapse, and reached the still lower level of 3.07 following the ingestion of a meal very rich in protein. The actual red cell count on the day of this experiment

NAME	CLINICAL DIAGNOSIS	AGE	URINE PICTURE	B. P. RANGE	EYE GROUND	ARTERIO- SCLEROSIS	SIZE OF HEART (cm.)		PHTHALEIN	NPN W/T		URINE VOL.		URINE UREA		INDEX	REMARKS	
							Rt.	Lt.		MG. 100 c.c.	KGMS.	BLOOD UREA MG. 100 c.c.	70 m. 24 hrs. Liters	70 m. 24 hrs. Grams				
1—Chalk	Pneumonia Convalescent	33	Sp. gr. 1010-1028 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	100/75-90/55	Negative	Slight	2.5	9	2 hours = 60	30.1	64	16.6	0.3	6.0	1.124	22.48	6.90	
2—Applebaum	Arterio- sclerosis	65	Sp. gr. 1015-1030 Casts 0. W.B.C. 0. Blood 0. Alb. trace	140/72	Vascular changes	Extreme	2	9	18+10=28	46.2	56.5	24.4	0.053	1.06	0.7925	15.85	8.39	
3—Keith	Tabes dorsalis	42	Sp. gr. 1018-1025 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	165/90-117/84	Negative	Slight	2.5	8.5		34.3	59	14.8	0.077	1.54	0.4898	9.796	6.94	
4—Talkington	Neurasthenia	43	Sp. gr. 1018 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	118/82	Negative	None	3	8.5		32.9	60.5	15.6	0.045	0.9	0.49156	9.831	8.54	
5—Sofnoski	Emphysema Myocardial insufficiency	57	Sp. gr. 1016-1040 Casts 0. W.B.C. 0. Blood 0. Alb. +	150/90-100/57	Negative	Moderate	5	9	65+20=85	40.0	69.5	25.2	0.05	1.0	0.70661	14.132	6.73	
6—Henderson	Elephantiasis Chronic nephritis	56	Sp. gr. 1010-1016 Casts +. W.B.C. +. Blood +. Alb. trace	120/66	Negative	Slight	3.5	13	30+5=35	36.2	103	14.0	0.18	3.6	1.10	22.0	8.17	
7—Block	Sub-acute endo- carditis. Strepto- coccus viridans	45	Sp. gr. 1018-1024 Casts 0. W.B.C. 0. Blood once. Alb. 0.	128/75-100/57	Vascular changes	Moderate	3	10.5	38+15=53	36.4	52	21.5	0.095	1.9	0.5355	10.71	5.02	
8—Leslie	Peritoneal carci- noma-tosis	58	Sp. gr. 1018-1021 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	190/95	Negative	Moderate	3.5	12	35+18=53	33.0	69.5	15.3	0.028	0.56	0.239	4.78	5.01	
9—Mitchell	Mitral stenosis Myocardial insuf. Chr. bronchitis	36	Sp. gr. 1018-1040 Casts 0. W.B.C. 0. Blood 0. Alb. trace	115/75-90/65	Negative	None	4	9.5	Unsuccessful	33.3	70.5	14.3	0.095	1.9	0.489	9.78	5.91	
10—Erdman	Lobar pneumonia Serum sickness	44	Sp. gr. 1010-1020 Casts +. W.B.C. +. Blood +. Alb. +	124/75-90/60	Negative	None	3	10.5	40+?	68		11.9	0.238	4.644	0.646	11.628	5.50	
11—Groeninger	Chr. bronchitis Bronchial asthma	51	Sp. gr. 1026 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	142/50	Negative	Moderate	2.5	9.5	25+10=35	33.3	49	16.4	0.238	4.76	0.894	17.88	7.16	
12—Cosgrove	Diabetes insipidus	52	Sp. gr. 1003-1009 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	175/95-149/80	Negative	Slight	3	10	35+35=70	28.6	73	11.5	0.23	4.6	0.44642	8.928	4.24	
13—Heinicke	Pernicious anemia	41	Sp. gr. 1011 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	114/64	Negative	None	4	9.5	35+20=55	23.3	73	16.7	0.109	1.962	0.528	9.504	4.74	Stationary low count.
14—Gibbons	Pernicious anemia	47	Sp. gr. 1015-1026 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	90/50	Negative	Slight	3.5	9	45+20=65	33.3	66.5	15.1	0.0355	0.71	0.1975	3.95	3.80	Stationary fairly low count.
15—Lucas	Pernicious anemia	36	Sp. gr. 1010-1018 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	110/50	Negative	None	3	II	2 hours = 30	36.2	59.5	18.2	0.122	2.44	0.474	9.48	4.33	Stationary active relapse.
										60	21.1	0.212	4.24	0.597	11.94	3.55		
										60	17.5	0.248	5.95	0.422	10.128	3.07		
16—Wilson	Chronic cardio- renal disease. In- dolent ulcers	53	Sp. gr. 1015-1030 Casts 0. W.B.C. few Blood 0. Alb. trace	190/115-140/85	Slight vascular changes	Moderate	3	13.5	38+15=53	38.7	75.5	24.7	0.05	1.0	0.4068	8.136	3.79	
17—Scheminant	Chronic nephritis	30	Sp. gr. 1010-1020 Casts +. W.B.C. 0. Blood 0. Alb. +	135/90	Negative	None	3	8.5	22+18=40	42.8	45.5	30.7	0.031	0.62	0.29365	5.873	3.60	
18—Stain	Hypertension Duodenal ulcer	35	Sp. gr. 1018-1030 Casts 0. W.B.C. 0. R.B.C. 0. Alb. trace	200/135-180/110	Slight vascular changes	Moderate	3	12	20+40=60	39.8	54	19.9	0.076	1.52	0.26251	5.24	2.90	
19—Wickers	Hypertension	37	Sp. gr. 1008-1024 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	180/124-130/94	Negative	Moderate	2.5	II	10+35=45	40.6	61	16.8	0.244	4.88	0.2918	5.836	2.02	
20—Stollar	Chronic hyper- trophic arthritis. Arteriosclerosis	65	Sp. gr. 1020 Casts 0. W.B.C. 0. Blood 0. Alb. 0.	180/105-138/78	Negative	Marked	2.5	10	2 hours = 28	26.7	74	12.4	0.086	1.72	0.1745	3.49	2.49	
21—Hynson	Chronic cardio- vascular-renal disease	53	Sp. gr. 1012-1030 Casts +. W.B.C. +. Blood +. Alb. +	235/136-208/78	Retinal arterioscle- rosis. Exudate. Few hemorrhages.	Marked	5	16	21+14=35	34.3	59	19.3	0.062	1.34	0.1894	3.788	2.21	
22—Levy	Arteriosclerosis. Arteriosclerotic ulcers	70	Sp. gr. 1022-1036 Casts occ. W.B.C. occ. Blood 0. Alb. 0.	155/78	Vascular changes	Extreme	3.5	10.5	14+18=32	29.5	72.5	17.8	0.042	0.84	0.2875	5.75	4.14	
23—Stewart	Chronic nephritis	43	Sp. gr. 1010-1018 Casts 0. W.B.C. 0. Blood 0. Alb. +	165/105-158/98	Negative	Slight	3	12.5	43+18=61	44.5	70	18.1	0.148	2.96	0.4855	9.71	3.73	
24 Metz	Sub-acute nephritis. Death	32	Sp. gr. 1029 Casts +. W.B.C. +. Blood +. Alb. +	170/90-178/86	Minimal retinal edema	None	3.5	9.5	Traces	341.0	79	150.0	0.005	0.1	0.0041	0.082	0.02	Dr. Darne
25—Webb	Sub-acute nephritis	18	Sp. gr. 1016-1021 Casts +. W.B.C. +. Blood +. Alb. +	140/78-107/50	Negligible	None	2.5	8	Traces	95.3	28	59.9	0.0265	0.53	0.0762	1.524	0.66	
26—Bazile	Chr. cardio-renal disease. Broncho- pneumonia. Death	52	Sp. gr. 1015 Casts +. W.B.C. +. Blood +. Alb. +	150/80-117/70	Retinal arterioscle- rosis. Massive exu- date. Many hemor- rhages.	Extreme	3.5	12.5	Traces	95.2	55	59.0	0.076	1.52	0.428	8.56	1.59	Dr. Oppen- heimer*
27—Leaby	Cholelithiasis. Obstructive jaundice	61	Sp. gr. 1020 Casts +. W.B.C. +. Blood 0. Alb. trace	195/98	Negative	None	3.5	10.5	30+5=35	35.7	90	21.1	0.011	0.22	0.0379	0.758	0.81	

* Necropsy:—Acute and chr. diffuse nephritis. Anasarca. Ascites. Bilateral hydrothorax. Pulmonary edema. Broncho-pneumonia. Acute splenic tumor. Scarred rt. apex. Caseous mediastinal glands. Phlebotitis of spleen and liver.

** Necropsy:—Chronic diffuse nephritis; arteriosclerosis; erosion and calcification of aortic valve with insufficiency and stenosis. Cardiac hypertrophy and dilatation. Acute bronchitis. Confluent lobular pneumonia. Acute splenic tumor. "Milk plaques" on heart. Old pleuritic adhesions.

was 1,780,000 and the hemoglobin reading was 37% (Sahli). Evidence of impaired renal function in severe anemias has been advanced by Mosenthal,⁴ and Christian has presented observations suggesting the same conclusion in a series of cases of pernicious anemia.⁵ However, as in Case 13, with a red cell count of 1,200,000, and a hemoglobin reading 25%, an index of 4.74 was found, the possibility of the participation of factors other than the severe anemia alone must be considered. This latter was a case in which the count had hovered just above one million for two weeks, with corresponding slight fluctuations in the hemoglobin readings. Case 14 at the time of observation presented a red cell count of 2,200,000, with hemoglobin of 38%, and was slowly losing ground. Coincidentally with the gradual decline in the count, a definitely low index was found. The inference is attractive that during the active relapses of pernicious anemia there is a definite impairment in the function of urea excretion, which shows definite improvement during the remissions of the disease, and that perhaps this impairment is not dependent solely upon the severity of the anemia.

The early detection of failing kidney function in hypertension is an absorbing problem. It is noteworthy in that connection that Cases 18 and 19, who present little evidence of functional impairment by the usual tests, yield indices comparable to those obtained from outspoken cases of chronic nephritis.

Except for Case 27, little comment upon the remainder is necessary. Even cursory scrutiny of the data will suffice to demonstrate the constancy with which marked renal damage is associated with a lowered excretory

index. In the case of obstructive jaundice, there is definite evidence of renal insufficiency, but the calculated coefficient is so low as to leave the impression that the collection of specimens was faulty. Yet a liberal allowance for error still gives a low index upon re-calculation, and it seems quite likely that in this case also there was a grave degree of kidney damage.

SUMMARY

Twenty-seven cases are presented in which the Austin-Stillman-Van Slyke urea excretory coefficient was determined. In borderline conditions, as well as in cases presenting marked evidence of renal damage, and also during the relapses of pernicious anemia, there was found a definite lowering of the value of the coefficient.

CONCLUSIONS

The Austin-Stillman-Van Slyke coefficient of urea excretion may be of value in demonstrating renal insufficiency, and, particularly in borderline cases, deserves further study.

The authors desire to acknowledge their indebtedness to Dr. William S. McCann for helpful interest and valuable suggestions.

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* FIG. 2.—Camera-lucida drawing of a cell containing pigment.

STUDIES ON EXPERIMENTAL RICKETS. XXII.

CONDITIONS WHICH MUST BE FULFILLED IN PREPARING ANIMALS FOR TESTING THE ANTI-RACHITIC EFFECT OF INDIVIDUAL FOODSTUFFS

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During the past two years we have carried out a study of the effects of faulty diets of various types on bone development. The results of this study have in part been reported in earlier papers.¹ Our observations have indicated that three dietary components are especially concerned in influencing the behavior of the several anatomic elements in the bones. These are calcium and phosphorus, and an organic substance which has as yet not been definitely characterized. We have recently

demonstrated that this substance is not identical with fat-soluble A. The relative proportions of these factors determine whether the animal will develop bones which are normal or bones which are pathological; whether the animal will have rickets, or osteoporosis, or an osteosclerosis.

Mellanby and we ourselves have shown that an animal must grow in order to have rickets. Given a diet which induces rickets, the more rapidly the animals grow the

more extensive are the lesions which are characteristic of the disease. When growth is but slight the rachitic metaphysis will be narrow, and the other characteristics of rickets will be poorly developed. This we have described in the eighth paper of this series.² The diet must be excellent except as regards calcium, phosphorus and the calcium-depositing substance, in order that maximal growth may be secured. The proteins of the food mixture should be of good quality. There should be a moderate amount of fat-soluble A (anti-xerophthalmic substance) available, and the diet should contain a satisfactory amount of water-soluble B, and the inorganic elements other than calcium and phosphorus. The calcium-depositing substance should be distinctly limited. Furthermore, as we ourselves, and Sherman and Pappenheimer,³ have shown, the quantitative relations between the calcium and phosphorus in the food supply is, within certain limits of concentration, of very great significance in influencing the development of the bones. The present paper deals especially with the effects produced on the anatomic structure of the bones by different ratios between the calcium and phosphate in the diet, other factors remaining essentially constant.

In a preceding paper of this series⁴ we described in detail a method for preparing young rats for the demonstration of the anti-rachitic effect of various substances in the diet.

In order to make this test in a satisfactory manner we have found it necessary to have the diet on which the animals are fed in the preparatory period of 25-35 days very nicely adjusted with respect to several factors. It is our purpose in the present communication to define more clearly the dietary conditions under which this test can be satisfactorily carried out, and to describe the lesions which are likely to be observed provided the composition of the diet used to prepare the animals for the test deviates, even to a relatively slight degree, from those which we have laid down.

Table I shows the composition of the diets discussed in this paper, together with their sources of additional calcium and phosphorus. In only two diets was the amount of phosphorus increased over that furnished by the organic components of the food mixtures. The table also shows the absolute amounts of calcium and of phosphorus in the diets, and the atomic and weight ratios between these elements. The effects of the several diets on the histological structure of the bones of young rats which were confined to them were characteristic. A description of the bones of animals confined to this series of diets makes clear how sensitive the rat is to changes in the composition of its diet of a magnitude which would have been considered as of no significance a few months ago. This series of experiments makes it clear that extremely careful work is required in studies on bone growth. A full appreciation of this fact will go far

toward preventing faulty interpretations by investigators who may undertake unsuccessfully to duplicate or extend observations in this direction.

METHODS OF SELECTING AND PREPARING FOODSTUFFS

There are several details of importance in experimental studies in nutrition which, it appears, some experimenters are likely not to appreciate, which we take this opportunity to emphasize. These relate especially to (1) the selection of vegetable food substances for feeding purposes; (2) the purification of proteins to be employed in supplementing the proteins from natural foods, or for supplying the total protein moiety in diets consisting essentially of purified food substances; (3) the determination of the composition of the mineral salts to be employed in feeding experiments; (4) the proper preparation of fats for supplying in experimental diets the desired amount of fat-soluble A; and (5) the proper evaluation of the vitamin content of certain extracts of natural foods employed as additions to food mixtures for experimental purposes.

1. We have always been very careful to select for feeding purposes only such grains as are free from disease. Any one familiar with seed grains knows that it is frequently found that the germinating power of grain is low or lacking. There are a number of causes for this, the chief ones being damage to the grains by unfavorable climatic conditions during the last stages of development, and infection of the kernels by bacteria or moulds. An inspection of many samples of maize, for example, will show that the germ or "heart" is darkened, owing to invasion by bacteria. In such seeds extensive decomposition of the food substances may have taken place, and even toxic products may have been formed. In other grains, badly developed kernels with low germinating power are the rule rather than the exception. While in the Wisconsin Experiment Station, it was easy to select grains which were sound. Since transferring our work to Baltimore we have for the above reasons secured our grains only from reputable seed dealers who handle grains which are known to be healthy and to have the power to germinate. Grains for nutritive studies should never be purchased from feed stores, since they will in most instances be inferior, and to some extent contaminated with other seeds, and great variation of quality is to be expected.

The history of leafy structures which are to be used for experimental purposes should be known, and they should be practically free from stems. They should not have been cured in the field unless the weather was exceptionally favorable for rapid desiccation. Leaching and the growth of bacteria and moulds must be avoided in their preparation. It is well known that sufficient salts leach from shocks of grains standing in the field to fertilize the soil to an extent that may greatly stimulate the growth of the next year's crop on these spots.

2. In the purification of casein two methods have been almost universally employed, and it seems to us that considerable confusion is likely to arise unless certain standards are adopted in the preparation of food materials. This is especially true in the study of experimental rickets. It is well known that when casein is precipitated from milk it takes with it very considerable amounts of inorganic salts. Whey, which is formed in cheese making through the action of rennet, is very poor in calcium, since this element is in the main carried down with the curd. It is not possible by any simple and easy method to purify casein in considerable amounts so as to make it suitable for experimental purposes.

During the last few years it has become a matter of some importance to use casein for feeding experiments which was free from vitamins, and a number of investigators have subjected casein, the purity of which was not stated, to continuous extrac-

tion with hot alcohol for the purpose of freeing it from fat-soluble A and water-soluble B. One is led by the comments of certain workers to conclude that they are of the opinion that exhaustion with alcohol is the most important treatment to which casein should be subjected in order to make a preparation of high quality for feeding purposes. It is difficult to see how this idea gained credence. Dry casein is impervious to alcohol, and extraction with alcohol is not likely to be effective in removing impurities incorporated within the casein granules. It is next to impossible to grind casein to an impalpable powder, and even if it is so ground, it is difficult to extract it effectively in a continuous extractor, because the material packs so tightly that the solvent does not permeate it uniformly, but finds paths of little resistance and tends to follow them, leaving much of the material incompletely acted upon. It is not to be expected that inorganic salts will be removed to any great extent by alcohol, so a casein preparation may be very laboriously extracted, and yet be little more suitable for use in nutrition experiments than before it was treated.

Our own method of purifying casein appears to have been little used by others except at the University of Wisconsin. The procedure has been described in a previous paper.⁵ It involves the handling of commercial casein in the following manner. The finely ground casein is placed in a tub and treated with a large amount of distilled water, to which acetic acid to the extent of about 0.2 per cent is added. About five pounds are handled at a time, and from eight to ten gallons of water are used. The first day the casein is washed by putting it on cheese cloth three times, and each time the greater part of the water is removed by moderate pressure on the cloth, after which the protein is returned to the tub and fresh water and acid are added. Thereafter for seven days more the change is made once a day. The repeated washing at first rapidly removes most of the sugar, soluble salts, and other contaminating substances on which bacteria would grow. The long continued washing with dilute acetic acid offers an opportunity for the soluble constituents to be extracted or to dialyse out of the granules. The acetic acid sets free the inorganic elements with which the acid groups of the casein molecule were combined, and these soluble acetates diffuse out of the granules and are washed away. Finally, the casein is soaked in water, drained, and then dried at about 75° C., and ground. This procedure results in a casein which is all but ash-free, and contains no demonstrable amounts of any vitamins. This method is, we are confident, much superior to that of extracting commercial casein with alcohol.

In selecting gelatin for experimental feeding, it is essential that only the highest grade be used, since the cheaper kinds contain, or are likely to contain, very considerable amounts of phosphorus and other inorganic elements. We have found Bactogelatin of the Digestive Ferments Company suitable, its only drawback being the high cost, which becomes a serious matter in extensive feeding studies. This we have analyzed and found to contain on an average about 0.120 per cent of phosphorus, and to be essentially free from other elements which might be of importance in influencing our results. Chemical analysis of gelatin should never be omitted in experimental work relating to rickets, and the small amounts of the significant elements which it furnishes should be taken into account in stating the content of any of these in the food mixtures.

3. Owing to the low ethical standards of certain manufacturers of inorganic salts which are widely used in chemical laboratories in this country, it is not safe to rely upon the label as evidence of chemical purity, or of the content of certain contaminating substances when these are stated, and purported to be based upon analysis by the manufacturer. There is much evidence to show that no labels are wasted, and that an analysis made at some time

in the past may be still serving as a basis of guarantee today. Salts should be purchased in large quantities if possible and a chemical examination of each purchase should be made before use.

4. For the preparation of butter fat we have regularly placed about eight pounds of first class creamery butter in a double boiler and allowed it to melt as quickly as possible. The water, salts, casein, etc., separate and settle to form a lower layer, while the fat forms an upper layer. This is decanted off upon a large folded filter supported in a hot-water funnel, the water in which is maintained at boiling temperature. The filtered fat is collected in pint Mason jars which are closed, and are stored away from direct sunlight.

5. It should not be necessary to point out that extracts of wheat germ, now so widely employed as a source of water-soluble B, may likewise contain considerable amounts of fat-soluble A. It is not warranted to introduce liberal amounts of such extracts into diets used to study the fat-soluble A content of fats, for the results may be entirely vitiated by so doing. Thus, an alcoholic extract of ten grams of wheat germ per 100 grams of food may add a considerable amount of fat-soluble A to a diet in which lard or cottonseed oil are being studied, and erroneous conclusions may be drawn from the results of feeding the mixture.

DISCUSSION OF EXPERIMENTAL WORK

In a foot-note to Table I the quality of the basal food mixture which served in these experiments has been sufficiently discussed. It should be borne in mind, however, that although the diets employed in these studies are fairly complex in their composition, each component has been the object of careful special study in our earlier work, and that the specific dietary properties of each is well understood. It is possible to predict from these special studies the results which will be secured from feeding mixtures of natural foods, with or without supplementary additions of purified food substances.

Although the most significant features of the results of feeding the experimental diets described have been noted under remarks in Table I, there are certain points which need further consideration.

The diet of Lot 3143 gives exaggerated rickets when administered to young rats. The histological structure of the bones induced by this diet has been described in another paper.⁵ We have made a special study of the extent to which slight modifications of this diet (our "line test" diet) interferes with the successful conduct of our "line test" for the calcium-depositing effect of any natural food. The following description of the histological characteristics of the bones of animals fed the series of diets described in Table I serves admirably to illustrate how very refined the experimental conditions must be maintained in order to avoid error in the conduct of this test.

If the diet of a young rat is modified so as to deviate from the composition of that of Lot 3143 in certain respects, distinct effects are observed in the histological picture of the bones (See Table I, Lot 3241). Under such conditions, even though the changes in the diet may

be of small magnitude, inconstancy of effects will be likely to occur and the data from a series of animals will appear confusing. To illustrate this the following modifications of diet 3143 will be described and their effects on the bones noted.

Diet 3241 produces changes which are identical with those seen in rachitic children. The epiphyseal disc of cartilage is much wider than normal and a rachitic metaphysis is formed between the cartilage and the shaft. The metaphysis is made up of a disorderly mass of osteoid tissue, connective tissue, blood vessels, and bone-marrow cells from the shaft of the bone. The trabeculae of osteoid are separated from each other by dilated blood vessels and delicate reticular tissue, which may or may not contain a few marrow cells. Where cartilage cells are in contact with metaphyseal blood vessels, the cartilage may be in all stages of metaplasia into osteoid. Many of the cartilage cells degenerate. On the other hand, in places the cartilage shows a much greater tendency to retain its individuality and staining characteristics than it does in the bones of rats fed on diet 3143. Long tongues of cartilage persist in the metaphysis, which retain the morphology and staining characteristics of the parent tissue. In other words, the tendency of the cartilage cells to metaplasia and to degeneration is less pronounced than it is in the bones of animals on diet 3143. The metaphyses are not quite so wide, nor are the ends of the bones as greatly enlarged as those of animals on the latter diet. The bones of many of these animals show traces of incomplete healing. These take the form in sections of more or less complete or fragmentary lines of calcified intracellular substance crossing the metaphyses of the bones. This calcified intracellular substance results from the reformation of the zone of provisional calcification which is commonly the first sign of healing in the rachitic bone. When relapses occur after remissions, the metaphysis goes on growing and the new proliferative zone is not immediately resorbed, but remains buried in the new osteoid tissue. Some bones show traces of as many as three of these lines of calcified cartilage. Bones which show this picture are apparently identical with those human bones which show relapsing or healing rickets. The trabeculae of the shaft are surrounded by broad zones of osteoid, but they are not more numerous than normal, and apparently little or no resorption of bone or osteoid occurs. The cortex is largely made up of osteoid tissue, and may be greatly thickened on one side. The marrow shows no change from normal, and there is no encroachment on the medullary cavity. The osteoid may or may not be lamellated, and the osteoid corpuscles are small.

These peculiarities of growth are due to the change in ratio between calcium and phosphorus in the diet. The ratio between these elements was nearer the optimum in this group (Lot 3241) than it was in that of Lot 3143, and the conditions were, therefore, more favorable to the production of normal bone, notwithstanding the shortage in the diet of the organic factor which favors the normal deposition of calcium. In Lot 3241, as in Lot 3143, the failure of the calcification was brought about by an excessive amount of calcium in the diet rather than by a deficit. The phosphorus content of both diets was essentially the same.

We may next consider the effects of reducing the calcium carbonate content of diet 3241 from 2 per cent to 1.5 per cent (See Table I, Lot 3228). This diet contained as nearly the optimal content of calcium as we can determine it at present. The phosphorus and the

organic factor concerned in calcium deposition were contained in the diet in amounts almost exactly corresponding to those of Lots 3143 and 3241 discussed above.

The bones of animals on this diet in general differed only very slightly from normal. The cartilage was narrow and well calcified. The cortex and trabeculae were slender, but were almost completely calcified. Only occasionally in the shaft was the border of osteoid tissue slightly wider than the physiological osteoid. There was some connective-tissue replacement of medullary tissue between the trabeculae of the spongiosa, and a few large cells with basophilic granules were found in the immediate neighborhood of the trabeculae. The cortex had a tendency to porosity in the neighborhood of the spongiosa. One of the animals on this diet showed the remains of calcified intercellular substance in the cortex as far down as the middle of the shaft of the bone. The trabeculae were not as straight as those of normal bones, and the trabeculae in the nucleus of ossification showed rims of osteoid tissue which were wider than those found in the normal animal. The bones of one animal, No. 1186, showed somewhat greater exaggeration of osteoid tissue than the rest of the series.

When we restricted young rats to diet 3227, which is the basal diet employed in this series, with no calcium additions, the bones were quite uniform in different animals.

This diet produced very imperfectly calcified bones. The cartilage in general was narrow and completely or nearly completely calcified. The trabeculae were small, convoluted and very numerous, as they are in the bones which show osteosclerosis. The trabeculae were incompletely calcified, and the calcium deposits in them had a granular appearance as though they were being rapidly disintegrated and rebuilt. They were partially or completely surrounded by zones of osteoid. A metaphysis was formed which had an orderly arrangement. The marrow was replaced between the trabeculae by young connective tissue containing many basophilic cells. The osteoblasts varied greatly in size and shape. The majority had a deeply stained uniform cytoplasm. The bone corpuscles were often surrounded by a relatively clear area which was bordered by a granular deposit of calcium. There were abundant signs of resorption present in the spongiosa. The cortex of the shaft was a lattice-like condensation of spongiosa. In the nucleus of ossification signs of resorption were wanting, and the osteoid tissue was broader than the osteoid borders of the trabeculae in the shaft.

The effect of modifying the ratio between calcium and phosphorus in our basal diet was further shown by certain experiments in which we have employed the same food mixture, Lot 3143 (see Table I), but with dicalcium phosphate added. This effect we may illustrate by Lots 3229 and 3173. Diet 3229 contained 2 per cent of calcium phosphate, which furnished the same amount of calcium as 1.5 per cent of calcium carbonate. Diet 3173 contained calcium phosphate equivalent to the calcium in 3 per cent of calcium carbonate.

The bones of animals in Lot 3229 were very well formed. The cartilage was somewhat narrow and was calcified throughout. The trabeculae were not quite so straight as those in a normal bone, and tended to be thicker. They were completely calcified, had no osteoid tissue about them, and were surrounded by a layer of flat osteoblasts. The space between the trabeculae was greater than normal and was filled with a delicate cellular connective tissue, which contained many basophilic cells and a few marrow elements. The cortex of the shaft was solid and very thick.

The center of ossification showed distinct porosis. The trabeculae of the center were few, thick and completely calcified.

The bones in Lot 3173 differed very little from normal. Trabeculae were more numerous and were straighter than in the bones of animals on diet 3229. Calcification was complete, save for the physiological osteoid tissue, which is evidence of normal growth.

We deemed it of importance to test the question whether several other calcium salts would yield comparable results in experiments of the character discussed. For this reason we tested the chloride and the lactate of calcium with our basal diet (See Table I). A rather surprising result was obtained with diet 3240, which contained calcium lactate 4.6 per cent (equivalent in its calcium content to calcium carbonate 1.5 per cent). Although this diet contained the same calcium: phosphate ratio as did diet 3228, the bones were not identical.

The bones of animals on diet 3240 were not abnormally thickened or deformed, but the histological picture was that of very mild rickets of low calcium type. The cartilage was abnormally broad, though the disc was not as wide as that which was produced by feeding diet 3142. It was incompletely calcified and had been invaded in places by blood vessels from the diaphysis, which had spared the calcified cartilage but invaded the rest of the disc. There was no definite wide rachitic metaphysis. The trabeculae of the shaft abutted directly in many places on the cartilage. In this situation they consisted of little more than spicules of calcified intercellular matrix or bits of calcified cartilage surrounded by narrow borders of structureless osteoid. The latter might contain very many large-sized osteoblasts crowded together almost to the limit of the capacity of the osteoid; or the osteoid might contain almost no cellular elements. The large osteoblasts were oval or polyhedral and their cytoplasm was uniform in shape and was not so deeply stained. The osteoid corpuscles lay in lacunae in the osteoid. In places the tissue in their neighborhood was infiltrated with sparse granular deposits of lime salts which did not enter the lacunae or encroach on the cytoplasm of the cell. Even at the epiphyseo-diaphyseal border of these bones the trabeculae showed granular deposits of calcium salts in their centers, which were the beginnings of ossification. The trabeculae farther down in the shaft were largely made up of mature bone. The osteoid tissue in the growing region was either lamellated or structureless. It was covered in places with a single or multiple layer of very large osteoblasts, or it was quite bare, or surrounded partially or completely with flattened osteoblasts of an endothelioid form. The trabeculae were separated by delicate reticular tissues, which supported numerous thin-walled blood vessels filled to capacity with blood cells. The connective tissues contained very few hæmatopoietic cells. A large number of osteoblasts lay free in it, and in some cases they were so numerous as to almost fill the space between the trabeculae. The trabeculae of the shaft were few in number. They were irregularly arranged and were surrounded by zones of lamellated osteoid tissue containing very few corpuscles. The osteoblasts which surrounded the osteoid in this region were of a flattened endothelioid type. The cortex was merely a lattice-like condensation of the spongiosa. The histology of the center of ossification corresponded to that of the shaft. In the growing region of the bone there were many signs of resorption as well as of active bone formation. Osteoblasts were fairly numerous but were not present in extraordinary numbers. The newly formed trabeculae showed many signs which can only be interpreted as evidence of bone destruction. Large numbers of perforating blood vessels were to be seen in these trabeculae in many places. In others

the osteoid tissue and the bone were being eroded by vascular loops from the diaphyseal circulatory system. In still other places newly built bone and calcified cartilage, partly stripped of its osteoid, seemed to be crumbling away under the influence of cellular and vascular activity. Here and there, large cells filled with basophilic granulations were to be found in approximation to the trabeculae. They were most numerous in the cortex and the older parts of the spongiosa. The bone marrow was apparently normal.

It will be seen that, in spite of the fact that the ratio between calcium and phosphorus was precisely the same in diet 3240 as in diet 3228, which with one exception produced animals with approximately normal bones, the bones of animals on diet 3240 were affected with a mild grade of atypical rickets. Moreover, the histology of the lesion would suggest that this rickets was a mild grade of the low calcium type. The sole difference between the two rations was that the calcium in diet 3240 was supplied in the form of the lactate, whereas that in diet 3228 was in the form of the carbonate. It is not possible at present to offer a satisfactory explanation for this difference in the relative effectiveness of the carbonate and lactate of calcium in promoting the development of the bones.

Lot 3236 contained 9.2 per cent of calcium lactate. This amount was sufficient to make the calcium content of the food equal to that of Lot 3143, which had 3 per cent of added calcium carbonate.

The bones of these animals were affected with very severe rickets, which corresponded exactly with the severest type commonly seen in children. At the same time, although the calcium-phosphate ratio in this diet was exactly the same as that in diet 3143, the bones bore a closer resemblance to those which resulted from the administration of diet 3241 (which contained 2.0 per cent of calcium carbonate) than to those which were produced as the result of feeding the former diet. The bones of animals of lots 3236 and 3241 differed from those of 3143 in that there was a greater tendency for the cartilage cell to retain its individuality and primitive characteristics in the former two. Moreover, on diet 3236, as on diet 3241, abortive attempts at healing with reformation of the provisional zone of calcification occurred, which left traces in the form of more or less complete lines of calcified intercellular substance in the cartilage and metaphysis of the bone.

Lot 3235 received calcium chloride 3.4 per cent (equivalent in calcium content to 3.0 per cent calcium carbonate). Animals on this diet developed very severe rickets.

The pathological picture of these bones was comparable to that seen in rats on diet 3143, except that the epiphyseal disc was slightly wider than that found in the bones of animals on the latter formula. There was a slightly greater tendency on the part of the cartilage cells to maintain their individuality and staining characteristics on diet 3235 than on 3143. Therefore, since the epiphyseal disc was invaded by numerous capillaries from the shaft, the epiphyseal disc had a very ragged margin on the metaphyseal side. Although the metaphysis of these bones was wider than the metaphysis of bones of animals on diet 3241, they were alike in that traces of abortive attempts at healing were found in the metaphysis. These were in the form of broken lines of calcified intercellular substance which were embedded in the osteoid of the metaphysis at right angles to the long axis of the bones.

TABLE SHOWING COMPOSITION OF DIETS AND THE NATURE OF THEIR DEFICIENCIES

No. of diet	Wheat	Maize	Gelatin	Wheat gluten	NaCl	CaCO ₃	CaCl ₂	Calcium lactate	CaHPO ₄	Ca in food	P in food	Wt. ratio Ca:P	Atomic ratio Ca:P	REMARKS
	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.	Gms.			
3143	33	33	15	15	1	3				1.2316	0.3019	1:0.2531	1:0.3163	This diet produces a very exaggerated form of rickets.
3241	34	33	15	15	1	2				0.8320	0.3059	1:0.367	1:0.472	This diet produces bones which are identical with those seen in rachitic children.
3228	43.5	33	15	15	1	1.5†				0.6320	0.3059	1:0.4840	1:0.6245	These bones in general differ only slightly from normal.
3227	36	33	15	15	1					0.0322	0.3101	1:9.630	1:12.430	These bones would seem to indicate that in this ration the phosphate tended to over-balance the calcium in its effects.
3229	34	33	15	15	1				2*	0.497	0.6668	1:1.3416	1:1.298	Bones well formed. Trabeculae not so straight as those of normal bone.
3173	33	32	15	15	1				4**	0.962	1.0277	1:1.0683	1:1.3718	Bones of these animals differ very little from the normal.
3240	31.4	33	15	15	1		4.6†			0.6303	0.2938	1:0.4661	1:0.6015	Histological picture is that of mild rickets of the low calcium type.
3236	26.8	33	15	15	1		9.2††			1.2305	0.2700	1:0.2194	1:0.2831	Very severe rickets corresponding to human type.
3235	32.6	33	15	15	1		3.4			1.2303	0.3019	1:0.2454	1:0.3166	Very severe rickets.

* Furnished calcium equivalent to that in 1.5 grams CaCO₃, and phosphorus 0.3609 grams.

** Furnished calcium equivalent to that in 3.0 grams CaCO₃, and phosphorus 0.7218 grams.

† Furnished calcium equivalent to that in 1.5 grams CaCO₃.

†† Furnished calcium equivalent to that in 3.0 grams CaCO₃.

‡ Contained 0.600 grams of calcium.

The quality of all the diets listed in the table was essentially identical as respected protein, fat soluble A, water-soluble B, and the uncharacterized substance which plays a rôle in directing the growth processes in the bones. None of these diets were capable of inducing growth at a rate faster than half the optimal for a period of a few weeks. This was due to deficiency of fat-soluble A, and except in the cases of diets 3229 and 3173 to lack of sufficient phosphorus. When these faults are corrected these food mixtures are all capable of inducing good nutrition in rats through successive generations.

We could not detect any influence of the chloride, which contains a non-oxidizable acid radical, in contrast with the carbonate or lactate, which are both potential neutralizers of acid in the body.

The results reported in this paper serve to extend and confirm our previous studies on the effect of deviating from the optimal ratios between calcium and phosphorus in the diet when the organic factor is low. They leave no room for doubt as to the importance of the relation between these two elements. The primary purpose in presenting these data was, however, to emphasize how one may readily fall into error in studying the anti-rachitic effect of any substance, unless the greatest care is taken to fulfill the conditions which we have so carefully standardized.

CONCLUSIONS

Dietary conditions are discussed which must be fulfilled in conducting our "line test" for the calcium-depositing substance (anti-rachitic effect) of foodstuffs.

The importance of selecting the highest quality of food grains; of using a rational system of protein purification; the use of salts of proven quality, and of taking into account the content of fat-soluble A in alcoholic extracts of wheat germ or other substance which may be employed

as sources of water-soluble B, are discussed and emphasized.

The pronounced anatomic changes which are brought about by relatively small variations in the composition of the diet, with respect to those factors which play a rôle in bone growth, are illustrated by specific examples from our experimental data.

The lactate of calcium proved a less satisfactory source of this element for promoting growth of the bones than did the carbonate. The cause of this difference must await further investigation.

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NOTES ON NEW BOOKS

Bibliography of the Writings of Sir William Osler, Bart., M.D., F.R.S. Regius Professor of Medicine in the University of Oxford. By MINNIE WRIGHT BLOGG, librarian of the Johns Hopkins Hospital.

This book is an amplification and virtual perfection of the bibliography of the writings of the late Dr. Osler, which appeared in the Johns Hopkins Hospital Bulletin in 1918. The original publication presented 730 titles, the number being increased later to 773 by titles subsequently sent by the author from England. The indefatigable compiler of the present volume, Miss Blogg, has now brought it up to 1195. The volume before us must, therefore, be considered as the definitive record of the writings of this remarkable man. The task has been an immense one and the thanks of medical men are due to her for her arduous labor. The varied character of Osler's writings is here characteristically shown. There are contributions on physiology, pathology and practical medicine, medical biography, the history of medicine, and reports of hospital practice and general medicine. There are also numerous text-books, the editions of which he so conscientiously revised for their progressive improvement, and monographs upon special subjects. Not the least interesting portion of the titles are the communications, notes and even editorials, which he supplied to the medical journals in which he was interested while a resident of Montreal, Philadelphia, Baltimore and Oxford. The wonderful industry and versatility of Dr. Osler is shown in the many additions which have been made to the original edition of this book. He was interested in every phase of medical knowledge and equally interested in all that pertained to medical education, and the contact between medicine and social service. The present edition of his bibliography is a monument also to the industry and painstaking work of the compiler. It should be on the desk of everyone interested in medical bibliography.

H. M. H.

The Diseases of Infants and Children. By J. P. CROZER GRIFFITH. (W. B. Saunders Company, Philadelphia and London, 1919.)

In presenting these two volumes the author has attempted to bridge the gap between the smaller text-books and a complete system of Pediatrics. The volumes are well edited and contain a moderate number of good illustrations. The author has drawn on his wide clinical experience and in addition shows a comprehensive knowledge of the native and foreign literature. The reader will profit by the numerous references which he gives and it is pleasing to note that he has referred to so many of the original articles on different subjects.

In discussing artificial feeding in infancy the author rightly emphasizes the point that there must be no fixed method of feeding. He shows that the "percentage method" and the "caloric method" of feeding are merely the outcome of certain

basic principles of nutrition. The two methods are really complementary and not diametrically opposed to one another, as has so often been imagined.

Except for two short chapters on infantile atrophy and malnutrition, the nutritional disorders of infancy are discussed primarily as diseases of the digestive system. Although it is true that many of the symptoms are referable to the gastrointestinal tract, the primary cause of most of these disorders is not a local lesion or functional disturbance but rather a disturbed tolerance of the whole body for certain food stuffs. Such conditions arise from many causes, as, for example, an improper balance in the food elements, overfeeding, and parenteral infection. In the last named the digestive disturbances are merely secondary complications. As a matter of fact we often see a profound nutritional disorder from improper feeding, with few or no digestive symptoms. If progress is to be made in this important subject a broader view-point than the author's must be adopted.

Apart from this subject the book is well handled. The chapters on systemic diseases are good and due consideration is paid to their peculiar characteristics in childhood. We feel that rheumatic fever might be considered under the infectious diseases, for although its etiology is still in doubt, it is certainly an infectious process. Bacillary dysentery should also be included under the same heading. Dr. Griffith doubts the value of the agglutination reaction in dysentery, although the majority of workers consider that this test, if properly done, gives a specific reaction in children, showing the presence or absence of infection with the dysentery bacillus.

One must take exception to the statement that *B. coli* is unable to grow in an alkaline medium. This has been shown to be incorrect. In his discussion of tetany the author writes: "The outcome is never fatal, although death may occur depending upon the primary disease or upon complications." However, he says that death may occur during attacks of laryngospasm occurring in this disease. As this is an integral part of the disease, we feel that his statement may give rise to misconceptions.

For students and practitioners the book may be recommended; in conjunction with more complete works on special subjects it will fill a place in the library of a specialist.

S. G. R.

The Evolution of Modern Medicine. By SIR WILLIAM OSLER. (London, New Haven, Oxford University Press, Yale University Press, 1921.)

Throughout the writings of Sir William Osler may be discerned an eager interest in the historical and bibliographic side of medicine. Steeped as he was in the classics and in their modern reincarnations, and endowed with the deepest instincts of the true literateur, it seemed all but impossible for him to write without apt allusion or allegory derived from

older sources. Among his works we find no less than 61 titles which may be said to deal purely with historical matters. It is altogether befitting, therefore, that we should have left to us one larger and reflective piece of historical writing from his pen.

"An airplane flight through the centuries" he calls it—and rightly—for men and events flash by; but there is no blurring, and when we close the volume the whole story is sharply scored on the tables of the mind. The idea of the origin of medicine from vague religious and social impulses is developed, then the first definite crystallization of the art with Asklepios and Hippocrates, and later its expansion under Galen, the dark days of the crash of the Western Empire, the lucky rescue of medicine from oblivion by the curious and indirect route of Arabia, while mediæval mysticism held sway in Europe, the Renaissance, and finally the rapid and inevitable developments of the modern era. But there is much more than the story of events. The story of ideas is developed with equal keenness. Osler has written not merely a chronicle but a piece of historical reflection of the very highest order.

Few readers in this day and age will read between the lines and grasp the real meaning of the book. It expresses Osler's creed—Grecian from start to finish—more directly stated in "The Old Humanities and the New Science." He realized well that despite material advance in science the road may lead not into Attica but to Bœotia. Never a reformer, he influenced men by precept and by example, and this History more than anything he has done stands for the broad and generous point of view in medicine.

A. L. B.

Acute Epidemic Encephalitis (Lethargic Encephalitis). An Investigation by the Association for Research in Nervous and Mental Diseases. (New York; Paul B. Hoeber, 1921.) \$2.50.

Despite the great importance of epidemic encephalitis the disease has not become common enough for any single observer's experience to be altogether comprehensive and authoritative. One welcomes, therefore, this collective investigation by such men as Barker, Hunt, Amoss, and Griffith, to mention only a few of the names of those concerned. The material of the book includes sound and critical summaries of the various facts which have been collected about encephalitis, each man presenting as it were "the evidence" in his domain of special interest. There is some question in the reviewer's mind as to whether the value of the book is enhanced by the verbal discussions at the end of each section which are reported verbatim apparently from extemporaneous remarks, and which perhaps carry the analogy to court proceedings a little too far. For the time being, however, this book must be regarded as a most valuable storehouse of information about an obscure and difficult disease.

A. L. B.

Oxford Medicine, Vol. V., Edited by HENRY A. CHRISTIAN and SIR JAMES MACKENZIE. (New York and London; Oxford University Press, 1922.)

Volume V. of the Oxford Medicine continues along the general lines of its predecessors. Interesting summaries of the infectious diseases are presented from a modern and satisfactory point of view.

A. L. B.

The Vitamins, by H. C. SHERMAN and S. L. SMITH. American Chemical Society Monograph Series. (New York, Chemical Catalogue Company, 1922.)

At a time when the lay mind is being actively stimulated to an interest in "vitamins," both by popular writers and manufacturers of various commercial vitamin products, it is very desirable to

have a review of the actual scientific knowledge of these substances, particularly of the sources from which they are readily and easily obtainable. To the physician, who is frequently besieged by questions on this subject, the concluding statement of the book is significant:—"We believe it is safe to say that with a dietary selected to make the best use of our ordinary staple foods there will rarely, if ever, be occasion to purchase vitamins in any other form, or to give any greater anxiety to the vitamins than to some other factors which enter into our present conception of nutritive requirements and food values." In the concluding chapter there is a useful table showing the distribution of "vitamins" in investigated food materials.

The bibliography comprises about one thousand titles. It is remarkable and very significant that the authors have been able to condense such an enormous literature into such a small space. To one who has watched the rapidity with which publications in this field have issued from the various laboratories, filled with hastily drawn conclusions and polemics, the task of making such a review is appalling. The authors have handled this difficult task without the introduction of personal bias. One feels that in some respects the review is not sufficiently critical.

It is of value for reference because of its unbiased point of view, and because it assigns to "the vitamins" no more than their proper share of importance in the broad field of the science of nutrition.

W. S. McC.

The Clinical Study of the Early Symptoms and Treatment of Circulatory Disease in General Practice. By R. M. WILSON, (Oxford Medical Publications, London, 1921.)

"You might just as well say, added the Dormouse,
.... that I breathe when I sleep is the same thing
as I sleep when I breathe."

ALICE IN WONDERLAND.

To those readers at all familiar with the author's views as expressed in his earlier work entitled *The Hearts of Man* many of the ideas set forth in this volume will come as no surprise, but to the uninitiated there will be much, we fear, that appears obscure in the elucidation of his unique hypothesis.

We confess at the outset that it was not without great effort that we were able to follow the author's frequently confused text and that our interest flagged tremendously long before the end came in sight. It would be equally false if we conveyed the impression that there are not a number of very sound truths contained in this volume. Unfortunately, many of them are lost in a bewildering maze of paradoxes that are indeed difficult to unravel and too often destructive of the author's purpose.

The *raison d'être* of the work is to set forth in great detail the author's theories of what we may call the balanced action met with in every physiological reaction, whether normal or pathological, and an attempt to analyze the effect of a stimulus, and the reaction following, upon the insecure basis of clinical observations alone.

A clue to the author's general attitude toward established physiological truths is offered early in his introduction when he says: "On the experimental table stimulation of the vagus slows the heart. But in clinical practice methods employed to this end *always* (italics ours) result in quickening it, even if a temporary slowing may at first occur!" The first five chapters devoted to the subject of Exhaustion constitute an attempt to apply the author's theories to the elucidation of a very complex problem due chiefly, he asserts, to the influence of pathological stimuli affecting primarily either the vagus or the sympathetic nerves. He postulates an extraordinary, one might say speaking paradoxically, almost human relationship between the vagus and sympathetic fibres frequently described as follows: "Thus to

borrow a phrase from the commercial world the cheques drawn by the vagus in its responses to stimuli are honored by the sympathetic." On page 39 he says: "Exhaustion, then, in a toxic state is first of all a matter of vagus excitability." A little further on "The toxic patient, then, presents a vagal picture (exhaustion) when and because reciprocal action by the sympathetic fails owing to depletion of that system. The patient with a mechanical disturbance in the viscera is in a different plight. The visceral disturbance causes sympathetic stimulation and so reflex vagal activity, and the latter soon dominates the former just as in response to outside stimuli the sympathetic when healthy soon dominates the vagus."

The chapter devoted to a study of Breathlessness is based upon a further application of the theory of vagal and sympathetic interplay. He divides breathlessness into two main groups, the "reaction type" of toxic origin due to action on the vagus, the "resting type" due to sympathetic exhaustion which in time may give place to a so-called "diaphragmatic type," when the respiratory rate increases and death is at hand. There is no discussion of the physiology of cardiac dyspnea, although allusion is made to the work of Haldane and Meakins and there is an occasional reference to oxygen want and oxygenation and the importance of the circulation.

The short section dealing with cyanosis is utterly unsatisfactory. All critical discussion of or even reference to the vast amount of work in recent years bearing on the subject is omitted. Referring to the incidence of cyanosis in pneumonia, its occurrence at the time of onset is over-emphasized and explained on the basis of an "invasion in mass of the toxin producing a very great vagus excitability to which the sympathetic could not at once respond."

The remaining chapters of the book are concerned with such topics as the symptoms of the patient's sensations, dealing with hyperalgesia, cardiac pain and headache, observations on blood pressure, the earliest symptoms of heart failure, and concluding with suggested treatment based upon early signs and symptoms; the whole discussed on the basis of the author's theory of the fundamental importance of the nervous system in relation to the subjective and objective evidences of cardiac disease.

The volume is introduced by a foreword from the pen of Sir James Mackenzie, in which he says that "Whether, then, the hypothesis which Dr. Wilson puts forward . . . is confirmed or disproved, he has rendered a service to medicine. Those who may cavil at such a work must bear in mind that here is but the beginning of a big undertaking." It is indeed difficult to understand the point of view which seems to have overtaken this distinguished observer in recent years. It is quite true, we admit,

that in clinical medicine two of the most important considerations "are the prognostic significance of a symptom and the mechanism of its production," but above all we need carefully controlled and interpreted observation. We cannot describe in too accurate detail our facts, but we should ever be cautious and over-critical in our conclusion based upon unsupported evidence.

E. P. C.

A Manual of Selected Biochemical Methods as Applied to Urine, Blood and Gastric Analysis. By FRANK P. UNDERHILL, XIV+ 232 pp. (New York: John Wiley & Sons; London: Chapman & Hall, Ltd., 1921.)

It is a distressing fact that biochemical methods of analysis are constantly changing and multiplying at a prodigious rate, and that manuals of such methods are short-lived undertakings. Yet Professor Underhill's compact volume presents to the biochemical analyst a more valuable and useful compendium than he has had hitherto. The great variety of the older and more modern methods of urine, blood, and gastric analysis are included, covering more than the ordinary routine procedures of a clinical laboratory, and furnishing ample substance for research work along these lines. A number of new and useful items for a manual of this kind are introduced, such as calibration of apparatus, acidimetry and alkalimetry, standardization of oxidizing solutions, etc. From the standpoint of instruction, the excellent plan of giving the principle involved in every analytical procedure has been faithfully followed. The different methods are mostly quoted verbatim from the original sources, perhaps too faithfully, with little attempt to clarify directions which are not clear or explicit enough in places. No critical appraisal is presented by the compiler on the comparative accuracy or simplicity of the methods, with the obvious presumption that each worker will choose the particular method which happens to suit his needs best. References being given in every instance to the original article, the reader is incited to study the method in which he is interested at greater length. The lack of tables comparing the content of urine and blood in normal and various pathological constituents as approximate guides in interpreting analytical results is also felt. Neither does the reader find any discussion of such debatable problems as the preferability of the use of whole blood or of plasma for the analysis of various constituents. It is hoped that this excellent manual will be revised from time to time so as to include the more valuable of the accretions to the biochemical methodology, and that it will have the wide use which it easily merits.

W. A. P.

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INTERFACIAL PHENOMENA

WITH ESPECIAL REFERENCE TO COLLOIDS AND ENZYMES*

By W. M. BAYLISS
(University College, London)

Although much attention is being given at the present time to the phenomena present in homogeneous solutions, and rightly so, it seems that there is need of more work on the mechanism of reactions in heterogeneous systems, and at the same time a clearer recognition of the fact that the majority of physiological processes take their course in such a system. Even when they appear at first sight to follow the relatively simple laws of mass action as applied to the conditions of homogeneous solutions, it must not be left out of sight that in the living organism they occur in the presence of various solid and liquid structures which may or may not have an important effect on their course.

For this reason, I think that considerations of some such cases form the most useful purpose to which I can

devote the first of these lectures, which I am enabled to give by the generosity of the Herter Foundation, to whom I wish to express my deep sense of the honour conferred by the invitation and the pleasure it gives me to put my views before so distinguished an assembly. I wish then to call attention to some points in the mechanism of physiological processes which appear to require further and more critical examination. In doing so, I would at the outset make it plain that I have the highest appreciation of the value of the work which has been done and, where it is necessary to criticize it, the criticisms are made with the object of pointing out the

* Lecture I of the Herter Series delivered before The Johns Hopkins University, March 7th, 1922.

risk of supposing that certain phenomena are already adequately explained, when the statement applies only to some aspects of them. Conditions peculiar to special cases are apt to be extended to a whole group of phenomena. This is particularly so when the colloidal state is involved. For example, a certain group of investigators would explain all the properties of this state on a purely chemical, others on a purely physical basis. It is scarcely necessary to remark that we want to know what is the actual state of affairs, and if we find that the physical state as well as the chemical nature of our systems has to be reckoned with, we base our conclusions on foundations liable to crumble away if we omit either of these.

It is no simple matter to define satisfactorily what is to be looked upon as a heterogeneous system. Perhaps it may suffice for our present purpose to say that it is composed of parts which, although in contact, do not mix together. These parts were named "phases" by Willard Gibbs, to whom we owe so much of our knowledge of the factors controlling equilibrium in systems of the kind mentioned. It will be clear that there are interfaces of contact where the phases meet and that the molecules forming these surfaces are exposed to forces which are not identical with those in the interior of the phases. For one thing, the molecules on the surface only are exposed to chemical action, so that the "active mass" is a function of the surface, not of the total mass present.

Some difficulty arises as to the dimensions required to claim the name of "phase." Single molecules, unless of very large size, cannot be possessed of the properties of surface, which imply the existence of a number of atoms joined together. The problem arises as to whether colloidal solutions obey Gibbs' "phase-rule," a point to which we shall return later.

What then are the most striking properties of surfaces as such? They are two, both conferring the possession of energy.

1. Surface tension, when one of the phases is liquid, and the corresponding property when we have only to deal with solids or gases. This phenomenon is due to the mutual attraction of molecules, giving rise to the "internal pressure" of Young and Laplace and to the "cohesive" forces of Matthews.

2. Electrical charge, which may be given in three ways:—

- (a) By the deposition of ions by adsorption, as investigated by Perrin. For example, ferric hydroxide may have either a positive or a negative charge, according as it is suspended in acid or alkaline media.

- (b) By electrolytic dissociation of the surface molecules. Thus, silica forms insoluble silicic anions and gives off hydrogen ions. The latter pass into the water, but only so far as electrostatic attraction to the opposite solid ion permits. The particles have thus a negative

charge, but are surrounded by a shell of positive ions in the liquid. Such systems have been called "electrolytic colloids" by Hardy; the large composite insoluble particle, consisting of unchanged matter with a layer of ions on the outside, is a "colloidal ion."

- (c) By electrolytic dissociation of whole molecules, with aggregation of one or other kind of ions resulting therefrom, the aggregated ion having a charge equal to the sum of those of its constituents. This happens in solutions of congo-red, as shown by my own work; in soaps, according to McBain, who regards the colloidal aggregates as consisting of anions, undissociated soap and water in various proportions. To these McBain gives the name "ionic micelle." The work of Sorensen and others shows that proteins behave similarly.

We may now proceed to discuss some special cases. Space forbids any great detail and I must be content with reference to points which seem to need further elucidation and to some in which my own work has played a part.

Turning first to the phenomena of adsorption, about which a variety of opinions still exists. While some have doubtless erred in attempting to reduce all the phenomena included under this head to changes in surface tension, on the other hand, there are facts which cannot be explained on a purely chemical basis. One of these latter was met with by myself. If we mix a dialyzed solution of the free acid of congo-red with colloidal aluminium hydroxide, free from alkali, a blue complex is precipitated which contains both the acid and the base, but not in chemical combination. They combine, however, slowly at room temperature, quickly if heated, to form the salt, which has the characteristic red colour of the congo-red dyes.

The problem concerns the nature of the forces which cause the attachment of one substance to the surface of another, without chemical combination being of necessity involved. The familiar formula of Gibbs, which gives the degree of adsorption in relation to surface tension, is based on thermodynamical theory. Like all such deductions, it can predict in what direction and to what extent a change of energy will occur, but it does not pretend to explain how this happens. In other words, we need in addition the assistance of molecular dynamics, and I think that it is wisest to admit that at present we have no adequate general theory of adsorption. In all probability, the mechanism is of different nature in different cases. While diminution of ordinary mechanical surface tension plays a part in the adsorption of some organic compounds by charcoal, for example, in the case of the congo-red acid and aluminium hydroxide, above mentioned, where we have to deal with electrically charged surfaces of opposite sign, there is no doubt that electrical forces are chiefly responsible. It is beside the point to urge the objection made by Matthews and to

exclude electrical adsorption on the ground that the combination of sodium and chlorine ions to form sodium chloride would be such a case. The fundamental difference is that in adsorption the chemical nature of the substances concerned is unchanged, whereas when ions unite in a true chemical way the properties are greatly altered. I may be allowed here to express a feeling that when chemists claim the numerous cases of association in which no change of properties occurs as being chemical in nature, they run some risk of obscuring the distinctive character of chemical phenomena.

Langmuir's view of adsorption is sometimes stated to be a purely chemical one. Although, in the case of the taking up of ions by the surface of some crystals, it is intelligible that unsatisfied chemical affinities of atoms in the boundary of the space lattice may cause the deposition of other appropriate atoms, it is not so easy to see how a fatty acid is combined with the surface of charcoal or carborundum. Moreover, as Bancroft points out, if adsorbed chlorine is in combination with the surface atoms of charcoal, it is difficult to understand why it is not an easy matter to obtain compounds of chlorine and carbon by direct union. On the other hand, since the carboxyl groups of acids have a greater affinity for water than the hydrocarbon residue has, it is easy to see why, after deposition on a surface, they are arranged with the former groups projecting into the water phase. But why the acids are in greater concentration at the interface than in the body of the solution is not explained hereby. It may further be remarked that Langmuir's view of adsorption implies that an adsorbed layer can never be more than one molecule in thickness. This is doubtless often the case, but there is also evidence that in some instances the amount adsorbed is in excess of that possible in a layer of the dimensions given. It appears as if the attractive forces extended their action somewhat further outwards than to the depth of one molecule. But such cases need careful investigation and whatever the truth may be, it must not be overlooked, as is sometimes done, that surfaces become saturated, sometimes after taking up only a very small amount of material. The well-known formula of Freundlich, indeed, which takes no account of this fact, applies only to a limited region of concentration. The existence of saturation, therefore, is not evidence that any particular case is not one of adsorption. Although it seems to me that Langmuir's view does not account fully for the facts of surface condensation, there is no doubt that, after orientation on the surface, chemical combination with the material of this surface or between different kinds of adsorbed molecules may occur. This is an important point in relation to the action of enzymes. It will be seen that appropriate orientation may facilitate mutual combination, or oppose it. Cases of the latter kind have been described by Van Krulyt.

To return for a moment to the phenomena of electrical adsorption. Michaelis and Rona have recently stated that the staining of paper by so-called "basic" dyes, that is, by salts which consist of a colourless acid in combination with a coloured base, is due entirely to chemical combination with salts contained in the paper. Although this statement is accepted by Prof. Matthews in his interesting article in *Physiological Reviews*, it is so directly in opposition to the work which I published some years ago in the *Biochemical Journal* and with experiments which are regularly made in my classes, that it is a matter of astonishment to me how skilful experimenters could have arrived at it. Since the actual facts are of interest in illustration of the phenomena of electrical adsorption, I may give a brief account of them. If filter paper is dyed with an acidic dye, such as congo-red, the purer the paper, the less is the dye adsorbed. On the other hand, with a basic dye, the purer the paper, that is, the smaller the amount of inorganic salt present, the deeper is the colour. A small addition of a neutral salt, even sodium chloride, has a powerful effect in increasing the depth of stain with the acidic dye, of decreasing it with the basic dye. This effect of electrolytes increases greatly with the valency of the cation. The explanation of these results is simple. Paper immersed in water has a negative charge, as shown by Quincke. The acidic dyes, if colloidal, have also negative charges and in any case the coloured anion is negative. Paper and dye are mutually repellent. Or, looked at from the point of view of energetics, if negative dye were deposited on negative paper, the magnitude of the charge would increase, which is contrary to the second law of thermodynamics. If the dye is positive, as the coloured cation of the basic dyes is, then mutual attraction between it and the negative paper occurs. Now suppose that a soluble inorganic cation, such as sodium, with a positive charge, is present. This will be deposited on the paper, partially neutralizing or abolishing its negative charge; the negative dye will be more easily adsorbed, the positive dye less so. There are various other phenomena which confirm this way of interpreting the facts, amongst others, the effect of inert liquids such as alcohol, which, by lowering the dielectric constant, lower the charge on the paper.

We may pass next to one or two problems concerning the large subject of colloids.

In all questions in which these substances play a part we must never forget that we have to deal with suspended particles of comparatively large size and therefore possessing interfaces of contact with the medium in which they are suspended, on which surface action of various kinds may occur.

The proteins are amongst the most important of the colloids. These, as composed of amino-acids, are "amphoteric" in nature, that is, they can combine with either strong acids or bases. At and near a certain small

hydrogen-ion concentration, called the "isoelectric point," they are free from capability of combination with either acid or base. Their physical properties, viscosity, osmotic pressure, electrical conductivity and so on, have minimal values in this region. So far as they owe their charge to electrolytic dissociation of their salts, they have none at the isoelectric point. A difficulty arises here if a charge can be conferred in any other way than by dissociation, say by adsorption of ions. In fact, as shown by Loeb, gelatin at the "isoelectric point" can be given a positive charge by cerium salts. What does the term "isoelectric point" mean here? It is a pure assumption to say that there is a "loose combination" between gelatin and cerium ions as between it and hydrogen-ions. In point of fact, although the term has a fairly definite meaning when used in relation to amphoteric compounds and hydrogen ions, there is a tendency to make it serve in cases where the state of affairs is altogether different. It was first introduced by Hardy in reference to the discharge and precipitation of arsenious sulphide by barium salts. Its application to the hydrogen ion alone is of later date. It must always be stated what ion is meant, since colloids are made isoelectric by various ions.

We have seen already that ions are adsorbed by surfaces and Perrin showed that the negative charge of cellulose could be nearly abolished by $M/20$ hydrochloric acid. Now in the case of proteins as amphoteric electrolytes, the hydrogen-ion concentration needed to make them isoelectric is a function of their relative strengths as acids and bases. If the isoelectric point of cellulose is to be interpreted in the same way, it would imply strongly acid properties. We must take into consideration the adsorption of hydrogen-ions, and the isoelectric point in this case means the concentration needed to abolish the negative charge which cellulose has in water, whatever may be its origin.

More objection must be taken to the use of the term in relation to the red blood corpuscles where the charge is due to the impermeability of the membrane for cations, while being permeable to anions. This will be considered in my next lecture. It is sufficient to point out here that this same property is responsible for the "Hamburger effect" of acids added to blood as increasing the apparent alkali reserve of the plasma.

It is a difficult matter in practice to find evidence of combination of the serum proteins with acid or alkali unless the hydrogen ion concentration is above 10^{-4} or below 10^{-9} respectively. It was suggested by Gustav Mann that uncombined proteins really exist in the form of internal ammonium salts, requiring a fairly high hydrogen or hydroxyl ion concentration to split the ring. But curves expressing the amount of combination with acid and base are very flat about the isoelectric region, so that it is not an easy matter to be certain on this matter. At all events, there is no appreciable combin-

ation between the limits named. In a series of experiments which I have made recently, proteins did not appear to behave towards sodium bicarbonate as such strong acids as their dissociation constants would imply. But there is considerable uncertainty as to their actual molecular weight. In the case of gelatin, however, all the acidic groups appear to be much more equally dissociated than is the case with ordinary di- or tri-basics acids.

A property of importance in regard to colloids in general is that it is practically certain that no single molecule or ion is sufficiently large to satisfy the criterion of not diffusing through parchment paper. At the isoelectric point Sorensen has shown that the Donnan equilibrium is unable to explain the low osmotic pressure of egg white and that there must be considerable aggregation. I have already referred to the cases of congo-red and soaps, in which the osmotic pressure in relation to the electrical conductivity is too low to be accounted for otherwise than on the assumption that the anions form aggregates. So that in any case either the undissociated colloid or its ions are aggregated. Now these aggregates must be large enough to possess surface properties, amongst others that of an electric charge of origin other than electrolytic dissociation of the molecules of the colloid themselves. In other words, a purely chemical theory cannot be the whole story, however satisfactorily an explanation of certain facts is afforded.

In many ways, the most interesting and important of colloidal substances met with in the animal organism is haemoglobin, and the various considerations which have been previously given have their application to it. The investigation is beset with many difficulties and the more work is devoted to it, the more seems to be necessary. I venture to think that at the present time too much attention is being given to the elaboration of details, interpreted on a limited theory, while there is yet uncertainty about many of the fundamental facts. For example, the isoelectric point as regards hydrogen ions is stated to be on the acid side of the reaction of blood, so that the question arises as to how far the preparations used were, wholly or in part, sodium salts. This possibility raises difficulties in deductions from the osmotic pressure measurements of Hüfner, which more recent determinations suggest were high for free haemoglobin. The question is of moment also in connection with the carriage of carbon dioxide by blood. Bohr stated that the preparations of haemoglobin on which he determined the carbon dioxide dissociation curve were free from sodium. Again, according to the acidic dissociation constant of haemoglobin, compared with that of carbonic acid, it would seem in any case that as much as 95 per cent of the available sodium is in combination with carbon dioxide. If this be so, theories of carbon dioxide carriage on the basis of interchange of sodium have little

support. But the actual molecular concentration of the acidic groups is uncertain. Perhaps the most pressing need is a method of preparing uniform samples of hæmoglobin, of known composition.

It will be remembered that the well-known equation of A. V. Hill was first put forward by him simply as a mathematical formula to express the experimental results obtained by Barcroft and his co-workers on the oxygen taken up by hæmoglobin at different pressures. A definite interpretation was soon, however, put upon the two constants. While expressing the greatest appreciation of the valuable collection of facts, experimental and clinical, made under the inspiration of this view, I must confess to an uncomfortable feeling that it may turn out that little more than the facts themselves stand future investigation. For example, in expressing by Hill's formula the oxygen dissociation curve as it is in the blood, it is found that the exponent, n , has to be made of a value between 2 and 3. If therefore it expresses the number of molecules taking part in the reaction, or the order of the mass action equation of velocity, it must be that there are a number of reactions of different molecular order in certain relative proportions. Now I am told by a friend who has looked at the problem from the mathematical standpoint that it is not admissible to take the index by itself and find the relative proportions of the various reactions required to make it of the value found (about 2.4). The equations themselves must be summated. This is a tiresome piece of algebra and seem to require the introduction of further constants to give determinate results. Moreover, it is remarkable that no direct evidence of the existence of such small aggregates in definite proportion has yet been obtained.

In respect to the numerous clinical estimations which have been interpreted on the lines of the Barcroft-Hill equation, the recent work of Lovatt Evans has shown that there is a rapid development of lactic acid in shed blood. This arises from the glucose and is prevented by the addition of 0.1 per cent of sodium fluoride. Since the presence of this acid has a marked effect on the dissociation curve of oxy-hæmoglobin, and also on the alkali reserve, some doubt is thrown on results obtained more than fifteen minutes after removal, unless sodium fluoride had been added.

It is a common experience to find that if acidified hæmoglobin solutions are dialyzed, with the object of obtaining freedom from sodium, methæmoglobin is formed with some rapidity. If 1 per cent of boric acid be added, it was found by A. V. Hill that this change is prevented and the hæmoglobin remains normal. Boric acid is a fairly efficient antiseptic against bacteria, but does not prevent the growth of moulds. The whole question of the relation of methæmoglobin to oxyhæmoglobin would repay further investigation, especially in connection with the manner of combination of oxygen.

Perhaps the former is the real *oxygen compound*, the latter something of another kind.

The validity of the phase rule as applied to colloids has its interest in the case of hæmoglobin. If it does apply, oxyhæmoglobin does not satisfy the requirements of a chemical compound. But it is to be remembered that Gibbs excluded from his treatment all factors except pressure, volume and concentration. Since surface energy and electrical forces intervene in the case of colloids, the matter is as yet undecided. Experiments might be made with saturated solutions of hæmoglobin in presence of a definite solid phase, say crystals, in which case the phase rule should hold. It is interesting to note that Cohn obtains evidence of a definite slight true solubility in the case of some proteins.

On the whole, it seems to me that the problem of hæmoglobin might be advanced if it were attacked from a quite independent point of view, taking account of possible effects of surface and without prejudice by previous theories. It is remarkable that the more careful the work with this substance, the more inexplicable it appears. As an illustration, I might refer to the recent researches of Adolph and Henderson on the heat of "combination" of oxygen and hæmoglobin, in which values varying from 1500 to 15,000 were given by a method capable of an accuracy of 1 per cent.

A few remarks are next suggested with respect to enzymes. Here there is no doubt about the fact that their action is exerted at their surfaces. The phenomena are indeed particular cases of catalysis in heterogeneous systems. It is easy to show that many enzymes are active in media, such as alcohol of moderate strength, in which they are quite insoluble and can be filtered off. This is especially simple with urease; powdered soy beans can be extracted with 70 per cent alcohol, the filtrate is inactive, while the residue suspended in alcohol of the same strength hydrolyses urea with rapidity.

It is evident also that adsorption of the reacting substances takes place on the surface of the enzyme phase and that a rapid equilibrium, brought about by some agency or other, is reached. Although the view that there is temporary formation of unstable intermediate compounds between the enzyme and the substrate on which it acts is a favourite one, it must be pointed out that the existence of such compounds, other than mere adsorption, has not been demonstrated. Whether close approximation between the reacting molecules is sufficient, as seems to have been the view of Faraday in the case of the combination between oxygen and hydrogen on the surface of platinum and other substances, or whether molecular forces brought into action raise the chemical potential, as suggested by Hardy; or again, whether the increased concentration raises the rate of reaction by mass action, remains as yet undecided. There is some difficulty in accepting the last view, if it be

supposed that the reaction occurs *after* adsorption because the molecules are fixed in position and the effect of mass is of course due to the movement of molecules. It may be, however, that the increase in rate of reaction occurs in the layer of concentrated solution close to the surface immediately before adsorption. The view of Langmuir that the reacting groups are brought into favourable position, for reaction with one another, by appropriate orientation on the surface, has already been mentioned. It must not be overlooked that acceptance of this view excludes the application of mass action formulæ and the existence of intermediate chemical compounds with the enzyme as explaining the phenomena. But these facts by no means put it out of court. With regard to the former, it is sometimes found that reactions in systems known to be heterogeneous obey, for a part of their course, a simple unimolecular formula. But surely this throws very little, if any, light on the nature of the process; it does not prove that it is a simple chemical reaction, any more than the increase of money at compound interest is. In many processes of the most varied kind, the rate of further change is proportional to what is left unchanged.

If the activity of an enzyme is proportional to the amount of the substrate adsorbed on its surface, it follows that if other substances are present which are themselves also adsorbed, a part of the surface is unavailable for the substrate itself and the rate of reaction will be slowed. I have investigated this in the cases of saponin and of amyl alcohol in their retarding effect on the rate of reaction in urease. It is of interest to find that the effect on the enzyme is merely to slow the rate of action, which ultimately results in the same total change. That the explanation as a case of competing adsorption is correct is confirmed by the fact that the effect has a negative temperature coefficient; that is, it is less at higher temperatures, a characteristic of adsorption phenomena.

The regarding of enzymes as being in the colloidal state suggests a way of explaining many of the effects of the addition of foreign substances, of heat and so on. These may change the degree of dispersion. If the particles become larger by aggregation, the active surface of a given mass is decreased and vice versa. Enzymes are usually destroyed by boiling, but sometimes not. It appears from the work of Chick and Martin on proteins that in the coagulation process the particles may sometimes merely stick loosely together and that they can then be redispersed by conferring an electric charge. In other cases, they fuse together to form a large single particle and redispersion is impossible.

Since the effect of an enzyme as catalyst is to accelerate the attainment of the equilibrium position when the reaction is a reversible one, it is clear that its effect may be either of a hydrolytic or synthetic nature according

to the conditions present. I have been unable to discover any evidence of the existence of an enzyme only able to effect a synthetic change, although statements of the kind have been made. The direction of the result produced by an enzyme in those cases where the action depends on the addition or removal of the elements of water, and these are the majority, is controlled by the concentration of water in the system. It is evident that in those frequent cases where the living cell exerts alternatively hydrolytic and synthetic action, say in the removal and storage of glycogen in the liver, there must be some mechanism by which the amount of free water can be changed as required. We naturally think of those colloids called emulsoids, whose particles contain different quantities of water according to the nature and concentration of the medium surrounding them. But there is also the possibility of adsorption of water on the surface. Probably both phenomena take place in the process of imbibition, the nature of which is not yet clear.

In connection with the equilibrium reached on the enzyme surface, there is another interesting problem awaiting investigation. If the constituents of a system are not adsorbed in the same proportion in which they exist in the natural equilibrium, it would be expected that the position of equilibrium under the action of surface catalysis would not be the same as the natural one or that produced by acid. In point of fact, certain cases have been reported where a difference of this kind exists, but whether it can be explained by differences of adsorption is unknown. I have been unable to find any change in the ethyl-acetate system on the addition of dried charcoal.

As a further case of adsorption, at all events as it appears to me, the cell-membrane or plasma-membrane may be considered. This is not to be regarded as a fixed permanent structure, but as produced by deposition of cell-constituents which lower surface energy at the interface between protoplasm and surrounding medium. Thus, it changes with cell activity and is in equilibrium with the cell contents as they alter. Thus, there is no difficulty in the membrane becoming permeable in the active state of the cell to substances to which it is impermeable in rest. Moreover, when a fresh protoplasmic surface is produced by mechanical action, a new membrane is naturally deposited on it. This is no doubt why large particles can be taken up in phagocytosis through a membrane which does not permit even sodium chloride in solution to pass. The particles actually break the membrane, which closes again behind them, in the same way as a needle can be passed through a soap film without bursting it, whereas a gas, such as hydrogen, nearly insoluble in the soap solution, only passes with extreme slowness.

It is not unlikely that a process akin to deposition of solid may occur in the surface membrane, similar to

that described by Ramsden with saponin, proteins and other substances. To account for its varying permeability, the structure suggested by the work of Clowes on phase reversal under the action of electrolytes seems to offer possibilities. A system of oil globules suspended in a watery protein solution can be converted into one in which drops of the watery solution are suspended in oil. It will be clear that a substance soluble in water but not in oil would be able to pass through the continuous watery phase of the former, but unable to pass in the latter case, since the only continuous phase is oil.

Finally, a word or two as to protoplasm itself. In its simplest form as in the clear pseudopodia of amoeboid cells, it is a liquid colloidal system or "sol." That it is colloidal is shown by the appearance of an immense number of particles under brilliant dark-ground illumination and that it is liquid is shown by the fact that

these particles are in Brownian movement. Under certain states of activity, it may become more jelly-like, its viscosity rises and the particles become fixed in a structure of some kind. I propose to refer to this matter again in the next lecture and for the present will merely call attention to the absence of any visible sign of structure whether of the nature of network, foam or otherwise. The production of such appearances by the action of fixing reagents was thoroughly investigated some time ago by Hardy, whose work has been rather unduly neglected by histologists. This action of fixing reagents is particularly striking in the case of the cell-body of the neurone, where Mott and Marinesco, independently, showed that there are merely granules or particles in Brownian movement in the living state. The Nissl bodies and neuro-fibrils are to be seen only after fixation, although of course the material out of which they are formed must be present in life.

FACTORS WHICH DETERMINE THE CONCENTRATION OF CALCIUM AND OF INORGANIC PHOSPHORUS IN THE BLOOD SERUM OF RATS

By BENJ. KRAMER and JOHN HOWLAND

There have been a number of experiments upon animals, in the past, with diets deficient in calcium. Most of these have been made in an attempt to produce rickets. The chief investigators, in this period, have been Voit,¹ Dibbelt,² Aron and Sebaner,³ Stelzner and Miwa,⁴ and Mellanby.⁵ The bones of the animals have been examined. In many instances the diet has been carefully controlled so far as the amount of calcium was concerned but, of the authors mentioned above, only Mellanby has paid attention to the other factors, such as the fat, in the diet. Mellanby has reported no determinations upon the blood. Aron and Sebaner fed to a dog a diet containing only .07 per cent calcium which in the light of our studies, assuming that the dog reacts to a deficiency of calcium in the diet as does the rat, should have produced a distinct lowering of the calcium in the blood. They found 6.5 mg. Ca per 100 gms. of whole blood, which is within normal limits. The diet, however, contained a large amount of beef fat (6 per cent) which, in the light of present knowledge, may account for the normal calcium concentration of the blood.

Experiments with diets deficient in phosphorus have also been made, chiefly by Schmori⁶ and W. Heubner.⁷ Neither reports analyses of the blood or serum. Within the past few years numerous attempts have been made to increase the calcium concentration of the blood by feeding or injecting calcium salts in large amounts. Among the investigators have been Heubner and Rona,⁸ Denis and Minot,⁹ von Fenyvessy and Freund¹⁰ and

Clark.¹¹ Analyses of the blood and serum for calcium have been made by various methods. The consensus of opinion is that while it is possible to increase the calcium concentration by the injection of large amounts of calcium, this increase is very temporary and there is a strong tendency for the concentration to be maintained within normal limits. The normal calcium concentration cannot be exceeded even when large amounts of calcium are fed.

Injections of solutions of inorganic phosphate have been made and a temporary increase of serum phosphorus demonstrated (Greenwald,¹² Iversen¹³), but there are no experiments, so far as we are aware, to show any permanent influence upon the inorganic phosphate of the blood or serum resulting from the injection or feeding of phosphorus in any form in excess.

We have attempted to study the factors that control the concentration of calcium and inorganic phosphorus in the serum of rats. The experiments of Dr. E. V. McCollum and of Miss Nina Simmonds, in which the various dietary ingredients have been altered one at a time, have enabled us to do this in a satisfactory manner. We are greatly indebted to Dr. McCollum and Miss Simmonds for the opportunity of making these studies and for the great assistance that they have afforded us, as well as for their enthusiastic cooperation.

The animals were fed and housed in the Department of Chemical Hygiene and are the same animals whose bones and tissues have been studied in the collective

investigation of rickets carried on by the Departments of Chemical Hygiene and Pediatrics.

There are two chief types of experiments. (1) Those in which animals were fed continuously upon the same diet, sometimes for a number of generations and (2) those in which the animals, after being on diets that were known to produce definite changes, were subjected to alterations in the shape of additions to the diet or to some physical influence.

In general the diets were made up so as to contain an excess of, or to be deficient in, some biologically important ingredient. To these diets were added various substances whose influence upon the concentration of Ca or P it was desired to study. By feeding several successive generations of animals upon the same diet it was possible to bring out, in some instances, deficiencies which were not readily apparent in the earlier generations. Some diets were so defective as to prevent reproduction. Others did not allow the animals to survive for even a reasonable length of time.

The appearance and behavior of the animals, the morphological changes in their tissues, especially the bones, have been or will be referred to by others. Our interest has been focused chiefly upon the alterations in the concentrations of Ca and inorganic P of the serum of these animals.

In order to establish normal values for the concentrations of Ca and Inorganic P in the serum we have made determinations of these substances on a large number of rats fed upon diets which allow normal growth through at least four generations and maximum longevity. We believe the normal to be for Ca 9.5 and 10.5 and for Inorganic P 7-8.5 mg. per 100 cc. serum.

There were two chief basal diets. The one was composed of wheat 30, maize 20, rice 10, rolled oats 10, peas 10, Navy beans 10, washed casein 10, the other of wheat 33, maize 33, gelatin 15, wheat gluten 15, NaCl 1, Ca Co₃ 3.* The first diet was deficient in calcium, the second in phosphorus. Each had sufficient anti-xerophthalmic factor but there was not enough fat of good quality to support normal growth. The proteins of each diet were satisfactory. When the additions (usually small) were made to these diets the proportion of some of the ingredients was necessarily somewhat reduced but not enough to disturb essentially the character of the diet.

The additions were designed either to restore to the diet the deficiency of calcium or phosphorus or to test the effect of certain agents, themselves not containing calcium or phosphorus, upon the calcium and inorganic phosphorus concentration of the serum.

The animals were studied in groups. There were control animals for every experiment usually from the same litter. Our results chiefly represent averages, because the animals of one group were sacrificed at the same

time and the blood pooled. Occasionally, if the animals were large enough, estimations were made from the serum of one animal and compared with the pooled serum. The blood was obtained by cutting the carotid arteries and jugular veins under complete anesthesia. The blood after collection was centrifuged at once and the serum removed. The serum was not allowed to stand in contact with the clot.

The calcium was determined by the method of Kramer and Tisdall¹⁴ and the inorganic phosphorus by that of Marriott and Hässler.¹⁵ The methods were frequently controlled by determinations on solutions containing known quantities of the respective element and by analyses of normal serum.

TABLE I.

Serial No.	Diet		Addition	Serum		Generation
	Ca	P		Ca	P	
633	.64	.40	B.F. 5	10.0	8.4	3rd
630	.65	.45	C.L.O. 2	9.8	8.0	1st
694	.05	.45	0	5.6	—	1st
696	.05	.45	0	5.9	—	1st
761	.05	.45	C.L.O. 0	5.5	8.0	1st
639	.18	.45	0	7.5	8.0	2nd
618	1.22	.30	0	10.5	2.4	1st
683	.09	.45	C.L.O. 2	6.8	8.0	3rd
656	.09	.45	C.L.O. 2	8.8	7.0	2nd
684	.13	.45	C.L.O. 2	10.2	7.8	2nd
666	.17	.45	C.L.O. 2	9.0	8.8	2nd
636	.17	.45	C.L.O. 2	9.4	8.0	2nd
635	.21	.45	C.L.O. 2	9.3	8.0	3rd
657	.21	.45	C.L.O. 2	9.1	8.2	4th
634	.25	.45	C.L.O. 2	9.7	8.2	3rd
658	.25	.45	C.L.O. 2	9.7	8.0	3rd
630	.65	.45	C.L.O. 2	9.8	8.0	1st
647	.65	.45	C.L.O. 4	10.5	—	2nd
652	.65	.45	C.L.O. 5	10.0	8.8	2nd
629	.65	.45	C.L.O. 5	9.8	8.2	2nd
651	.65	.45	C.L.O. 5	10.0	8.0	2nd
648	.65	.45	C.L.O. 7	10.2	—	2nd
639	.18	.45	C.L.O. 0	7.5	8.0	2nd
638	.18	.45	C.L.O. 1	9.6	8.0	2nd
707	.09	.42	S.L.O. 3	9.8	8.4	2nd

C.L.O.—Cod-liver oil.

S.L.O.—Shark-liver oil.

B.F.—Butter fat.

Figures for Ca and P in diet expressed as gms. per 100 gms. of diet.

Ca and P in serum expressed as mgs. per 100 c.c. of serum.

In the second and third columns of the table are given the amounts of calcium and phosphorus in 100 gms. of the diet; in other words, the percentage composition as regards these ingredients. Column 4 states the nature and amounts of the substances added to the basal diet. In the fifth and sixth columns are given the concentrations of the calcium and inorganic phosphorus expressed in the terms of milligrams per 100 cc. of serum.

It will be seen that if the diet contains calcium about .6 per cent and phosphorus .4-.5 per cent, and if the ani-

* These figures represent gms. per 100 gms. of diet.

mal receives butter fat 5 per cent or cod-liver oil, 2 per cent, normal values for Ca and inorganic P are obtained in the serum even for several generations. If the phosphorus is maintained at the same level but the calcium reduced to .05 per cent, and if no fat is added, there is a marked decrease in the calcium of the serum, the phosphorus remaining normal. An increase in the calcium of the diet raises the calcium of the serum even without the addition of any organic factor.

A most striking effect upon the calcium results from cod-liver oil administration. Even with so low a calcium intake as .09%, cod-liver oil, 2 per cent, added to the diet, raises the calcium of the serum to 8.8 in the second and to 6.8 in the third generation. Such a diet permits reproduction for at least two generations. As the calcium intake rises there is, in general, an increase in the calcium of the serum and when the diet contains only 0.21% calcium, the calcium of the serum is maintained at a nearly normal level for four generations. When the calcium of the diet reaches a certain concentration the addition of more calcium, with or without cod-liver oil, even in large amount does not further raise the calcium in the serum. This is shown in Table I and also in other tables. Determinations 639 and 638 in Table I illustrate several facts. A diet slightly deficient in calcium, containing no added fat but a normal phosphorus concentration, can bring about reproduction at least of one generation. That the diet is defective is indicated by its inability to maintain a normal calcium concentration. When to this diet so little as 1 per cent cod-liver oil is added a normal calcium concentration of the serum is found even in the second generation.

Determination 707 shows that shark's-liver oil is also very effective in maintaining a normal calcium content in the serum despite a low calcium content of the food.

TABLE II.

Serial No.	Diet		Addition	Serum		Generation
	Ca	P		Ca	P	
761	.05	.45	0	5.5	8.0	1st
708	.05	.45	C.L.O. 2% for 6 days	6.5	8.0	1st
762	.05	.45	C.L.O. 2 for 14 days	8.2	7.0	1st

Table II shows the influence of cod-liver oil upon the calcium concentration of the serum of animals receiving very small amounts of calcium. The animals were placed upon the diet without cod-liver oil when about twenty-five days old. They had been on the diet twenty-one days when the oil was added. No 761 represents the control animals of the same age. Cod-liver oil shows its influence in six days and in fourteen days the calcium of the serum has reached nearly normal limits.

A number of facts are illustrated in Table III.

TABLE III.

Serial No.	Diet		Addition	Serum		Generation
	Ca	P		Ca	P	
649	.05	.45	C.L.O. 3	9.7	—	2nd
650	.05	.45	C.L.O. 3	6.1	—	3rd
664	.13	.45	B.F. 8	4.4	8.0	3rd
624	.25	.45	B.F. 3	10.0	8.0	3rd
627	.21	.45	B.F. 8	9.4	8.0	4th
684	.13	.45	C.L.O. 2	10.2	7.8	2nd
656	.09	.45	C.L.O. 2	8.8	7.0	2nd
683	.09	.45	C.L.O. 2	6.8	8.0	3rd
635	.21	.45	C.L.O. 2	9.3	8.0	3rd
657	.21	.45	C.L.O. 2	9.1	8.2	4th

Determinations 649 and 650, 656 and 683 show the influence of generations. In both sets the phosphorus of the diet was adequate; the calcium was very low. Cod-liver oil was added to the diet. This was effective in maintaining the calcium of the serum at a normal or nearly normal level in the second generation but was unable to accomplish this in the third. Determinations 635 and 657 show that when the calcium of the diet reaches about .2 per cent and cod-liver oil is given, the calcium is maintained at a normal level even throughout the fourth generation. Determinations 664 and 627 show that a very slight increase of calcium has a profound influence on the calcium of the serum so that, when the calcium of the diet is increased only .08 per cent, the amount of butter in the diet remaining the same, the calcium concentration of the serum is maintained at a normal level even in the fourth generation, while without this increase the calcium concentration is less than half the normal even in the third generation. When the calcium of the diet is increased to .25 per cent, the calcium of the serum is maintained at a normal level in the third generation in spite of a reduction of butter fat to 3 per cent.

That cod-liver oil has a far greater effect upon the calcium of the serum than butter fat is shown by Nos. 650 and 664. In spite of the fact that one diet contains .08 per cent more calcium than the other and 8 per cent butter fat, the diet with 3 per cent cod-liver oil maintains the calcium of the serum at a much higher level than does the diet containing butter fat.

All the animals represented in Table IV were fed on a diet high in calcium, low in phosphorus and containing just enough anti-xerophthalmic substance to protect the eyes. The animals were placed on the diet at about forty days of age. At this time they usually weighed about 50 grams. They were fed on the diet for from twenty-five to forty days depending upon the condition of the animal. At the end of this time some of the control animals were sacrificed. To the diet of others, substances were added whose influence it was desired to investigate. A few animals were given water but no food for a period of three days and were then sacrificed.

TABLE IV.

Serial No.	Diet		Addition	Serum		Generation
	Ca	P		Ca	P	
618	1.22	.3	0	10.5	2.4	1st
660	1.22	.3	0	10.0	2.8	1st
749	1.22	.3	0	9.8	2.8	1st
675	1.22	.3	0	—	3.0	1st
822	1.22	.3	0	10.2	2.0	1st
668	1.22	.3	C.L.O. 2% 5 days	10.3	5.7	1st
736	1.22	.3	B.O. 2% 6 days	—	3.8	1st
745	1.22	.3	B.O. 2% 10 days	—	4.5	1st
768	1.22	.3	S.L.O. 2% 10 days	—	6.0	1st
769	1.22	.3	M.O. 20% 15 days	10.1	2.7	1st
770	1.22	.3	O.O. 20% 15 days	9.9	2.4	1st
653	1.22	.3	B.F. 30% 10 days	10.2	4.4	1st
640	1.22	.3	B.F. 30% 14 days	—	7.0	1st
673	1.22	.3	B.F. 15% 12 days	—	4.8	1st
803	1.22	.27	0	9.4	2.0	1st
807	1.22	.32	0	10.0	2.0	1st
808	1.22	.30	0	—	2.6	1st
809	1.5	.3	—	10.0	1.8	—
674	1.22	.3	Starva- tion 3 days	—	8.6	—

B.O.—Burbot-liver oil.
M.O.—Maize oil.
O.O.—Olive oil.

Upon this basal diet, with great uniformity, the calcium concentration of serum is maintained at a normal level, while the inorganic phosphorus varies between 2 and 3 milligrams. First generation animals alone are included in the table because reproduction upon this diet is impossible.

All the fish-liver oils employed and these include those from the cod, shark, and burbot (freshwater) raise the inorganic P concentration of the serum. This effect is the more pronounced the longer the period of administration. The fact that normal figures were not reached was probably due to the short period of administration. The vegetable oils (maize and olive), even when administered in large amount, have no demonstrable effect. Butter fat occupies an intermediary position between the liver and vegetable oils. It has a distinct effect upon the phosphorus but it must be offered in larger amounts and for a considerable period of time.

When food is withheld for three days a rapid and remarkable increase of the inorganic phosphorus takes place. The inorganic phosphorus concentration of the serum rapidly reaches the normal level.

TABLE V.

Serial No.	Diet		Addition	Serum		Generation	Radiations from
	Ca	P		Ca	P		
749	1.22	.3	0	9.8	2.8	1st	
663	1.22	.3	0	10.3	3.5	1st	U. V. Ferric chromium condenser spark.
646	1.22	.3	0	—	4.5	1st	U. V. Ferric chromium spark.
662	1.22	.3	0	10.2	5.4	1st	U. V. Cadmium spark.
669	1.22	.3	0	10.2	5.7	1st	U. V. Cadmium spark.
671	1.22	.3	0	—	2.7	1st	Cadmium spark filtered through glass.
716	1.22	.3	0	10.4	4.1	1st	U. V. Mercury vapor quartz lamp.
733	1.22	.3	0	9.6	3.2	1st	Ozone.

Table V shows the effect of certain physical agents upon the inorganic phosphorus of the serum. The diet was the same as that given to the animals represented in Table IV, *i.e.*, one which contained an excess of calcium and a deficit of P, so that while the calcium of the serum was at a normal level the inorganic phosphorus of the serum was invariably low. The animals were given the basal diet, beginning at about four weeks of age, for a period of twenty-three to thirty-eight days.

They were then exposed to radiations emanating from various sources. Spectroscopic analysis of the rays was made in each instance to determine the presence or absence of the ultra-violet. The mercury vapor quartz lamp yielded rays which gave, among others, a band in the region of the second ultra-violet. A similar but more intense band was obtained with the cadmium spark. The inter-position of a plain glass filter blocked the second ultra-violet rays while it allowed the passage of a part of the first ultra-violet. The ferric chromium condenser spark gives rays which are rich in the first ultra-violet but poor in the second ultra-violet.

It will be seen from the table that following radiation by the mercury vapor quartz lamp, by the cadmium spark and by the ferric chromium condenser spark there is a definite increase in the inorganic phosphorus of the serum. The only exception is when the rays are filtered through glass. Following this there is no demonstrable effect. The rays from the ferric chromium condenser spark are the least, those from the cadmium spark the most effective; those from the mercury vapor quartz lamp occupy an intermediate position. In all of these tests the time of exposure varied from fifteen to thirty minutes. The animals received ten treatments. The distance from the source of light was six inches. As there is a rapid formation of ozone by the mercury vapor quartz lamp a group of animals have been exposed to ozone for the same period and frequency as when radiations were employed. One of the animals had refused food for several days. The slight increase of inorganic phosphorus was probably the result of pooling the serum

of this starving animal with that of the others. The increase over that from the basal diet in any event was not significant.

TABLE VI.

Serial No.	Diet		Addition	Serum		Generation
	Ca	P		Ca	P	
749	1.22	.3	0	9.8	2.8	1st
782	1.22	.47	Cas. 20% for 10 days	10.4	4.4	1st
681	1.22	1.21	CaH ₂ PO ₄ 4% for 10 days	5.0	7.2	1st
842	.05	.45	0	4.4	8.0	1st
846	.05	.56	NaH ₂ PO ₄ .425%	4.5	7.2	1st
843	.05	.66	NaH ₂ PO ₄ .85%	4.5	—	1st
847	.05	.87	NaH ₂ PO ₄ .1.7%	—	8.0	1st
841	.05	.95	NaH ₂ PO ₄ 2.1%	4.2	—	1st
844	.05	1.06	NaH ₂ PO ₄ 2.5%	4.1	8.6	1st

In Table VI are shown results obtained with animals fed upon both types of basal diets. The diet of some animals was high in calcium and low in phosphorus. The phosphorus was increased by the addition of casein or of secondary calcium phosphate. Both of these substances raised the inorganic phosphorus of the serum, but whereas the casein had no effect upon the calcium, the secondary calcium phosphate reduced this by fifty per cent, in spite of the large amount of calcium in the diet.

The second diet was very low in calcium and contained an adequate amount of phosphorus. The control animals on this diet have regularly from 4 to 5 mgms. of calcium with a normal phosphorus concentration in the serum. The addition of acid sodium phosphate to this diet in increasing quantity produced practically no effect upon the concentration of calcium or phosphorus. There was a slight tendency for the calcium to diminish and the phosphorus to increase in the serum with the larger additions.

SUMMARY

A study has been made of the influence of various factors upon the concentration of calcium and inorganic phosphorus in the serum of rats. The concentration of neither element has been distinctly increased beyond normal limits, either as the result of various additions to the diet or the use of physical agents. It seems fair to assume that this cannot be done by either of these methods. On the other hand, it is possible to reduce the concentration of these elements in the serum by feeding diets containing an insufficient quantity of the respective elements.

In order to bring this about there must be only enough of the organic factor represented by cod-liver oil or butter fat to prevent xerophthalmia and allow moderate growth. Even with a very small amount of calcium or phosphorus in the diet, compensation occurs and the concentration of these elements in the serum approaches the normal level if sufficient of the organic factor is included in the diet. A defect in the diet is accentuated in succeeding generations, so that a diet which gives a normal value for the serum calcium in the second generation may give a distinctly low figure in the third generation. Of the fats that have been employed in these experiments the fat from the livers of fish has been much more effective than butter fat. The vegetable oils (olive and maize) have been without influence.

When the concentration of either element in the serum is low as a result of a deficiency in the diet, it may be increased by increasing the amount of the respective element in the food. Up to a certain point even small additions to the diet have a pronounced effect upon the serum. Further additions have no effect whatever if a normal concentration has been reached.

When the diet is defective in phosphorus and the phosphorus of the serum is therefore low, a marked increase of the serum phosphorus may be produced by starvation, by the addition of phosphorus to the diet in organic or inorganic form, by various fats, and by exposure to radiations from various sources which yield, among others, rays whose wave lengths are less than 3000 Aengstrom units.

The relation of these findings to rickets and to calcification in general will be discussed in a subsequent communication.

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A STUDY OF THE RESULTS OBTAINED IN SIXTY-FOUR CAESAREAN SECTIONS TERMINATED BY SUPRAVAGINAL HYSTERECTOMY

By JOHN W. HARRIS

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Eardley Holland¹ has recently published a statistical study of 4197 Cæsarean sections which were done in Great Britain during the decade from 1911 to 1920, and which in a way formed a continuation of a similar study by Armand Routh² for the previous decade. He stated that in the series there were forty-six Cæsarean sections which had been terminated by supravaginal hysterectomy with eight deaths, a percentage of 17.4. As this is far in excess of the mortality in our service, it seemed that it might be interesting to study the results which we have obtained and to compare them with those reported by Holland.

From the early days of our service it was noted that the convalescence following supravaginal amputation was more satisfactory and much smoother than that following most conservative sections, with the result that we gradually came to employ the former operation whenever it seemed justifiable, more particularly in two classes of cases;—First, as the method of choice for effecting sterilization after repeated Cæsarean section, and secondly, as a means of obtaining more ideal results upon patients who were already infected or who were admitted to the service after being long in labor.

In the earlier days of the service many conservative Cæsarean sections were done in the second stage or late in the first stage, with the idea that the patient should be given a test of labor in order to ascertain whether the head would or would not engage. We gradually learned that this was an erroneous practice and resulted in a relatively high mortality. After this we limited the performance of conservative Cæsarean section practically to such patients as could be operated upon at an appointed time before the onset of labor or as soon as possible after the appearance of labor pains; while in patients who were seen late in labor or who already presented signs of infection, we either did not do a Cæsarean at all or removed the uterus after its performance. Some idea of the improvement of the results which followed may be gained from the fact that in the first fifty Cæsarean sections done in the service the mortality was twelve per cent, while in the following 173 operations done up to May 15, 1922 it became reduced to 2.88 per cent.

In 223 Cæsarean sections which have been done in the obstetrical service of the Johns Hopkins Hospital up to May 15, 1922 the uterus was removed by supravaginal hysterectomy in 64 instances. For purposes of study we have divided our 64 cases into two main groups—the first

including all patients from whom the uterus was removed at the first Cæsarean section, and the second those from whom it was removed at the second or third section, as shown in Tables 1 and 2. The indications, which were discussed in detail in Dr. Williams' paper "A Critical Analysis of Twenty-one Years' Experience with Cæsarean Section,"³ covered our material up to the end of 1921.

TABLE I.
Supravaginal Hysterectomy at First Cæsarean Section.

Late in Labor or Manifest Infection.....	10
Sterilization.....	5
Heart Disease.....	5
Atresia of Cervix.....	5
Neglected Transverse Presentation.....	4
Myoma of Cervix.....	3
Hour-glass Contraction of Uterus.....	2
Apoplexy of Uterus.....	2
Failure of Bag or Bougie.....	2
Uncontrollable Hæmorrhage.....	2
Dystocia following Ventral Fixation.....	1
Total.....	41

TABLE II.
Supravaginal Hysterectomy at Repeated Cæsarean Section.

Late in Labor or Manifest Infection.....	8
Sterilization.....	13
Tearing of Uterine Incision.....	1
Blocking of Outlet by Condyloma.....	1
Total.....	23

Our figures show that in the sixty-four patients there were three deaths, a gross mortality of 4.68 per cent, which is, roughly speaking, only one quarter as great as that reported by Holland. As will be seen below, none of the deaths were due to infection and only one of them was associated directly with the operation. The latter occurred at a second section upon a multiparous patient with a generally contracted pelvis, from whom the uterus was removed for the purpose of effecting sterilization. Owing to the inexperience of the operator difficulty was experienced in ligating the left uterine artery, with the result that death from hæmorrhage occurred on the operating table.

The other two deaths were not connected with the operation and occurred under the following circumstances. The first was in a seventeen-year-old primipara,

who came into the service on account of acute ulcerative endocarditis with broken compensation. As she was steadily growing worse, and as it was felt that the abdominal enlargement was aggravating her condition, it was decided to empty the uterus. It appeared that this could be accomplished most conservatively by Cæsarean section, and as it was furthermore felt that future pregnancies should be avoided, the uterus was removed by supravaginal hysterectomy. Death occurred on the eighth day from the cardiac condition. At autopsy no sign of wound infection was present, but an acute thrombo-endocarditis with perforation of the aortic valves was found.

The second patient was a multipara who had been under treatment for several months for hypertension not associated with albuminuria. At the eighth lunar month she began to bleed as the result of premature separation of the normally implanted placenta. A Vorhees bag was introduced which controlled the bleeding, but failed to dilate the cervix. Ten hours later the patient became alarmingly worse with rapidly increasing stupor, marked increase in the blood pressure and the presence of fifteen grams of albumin per liter in the urine, from which it previously had been absent. Under the circumstances it was felt that the only chance for recovery lay in immediate delivery and that this could be accomplished most satisfactorily by Cæsarean section. Upon incising the uterus we found the placenta completely separated and lying free in the uterine cavity, which contained several hundred cubic centimeters of blood. Hæmorrhage had also occurred into the walls of the uterus and had led to such disassociation of its muscular fibers that contraction could not occur. As the organ remained soft and flabby in spite of the administration of pituitary extract and manual irritation, supravaginal hysterectomy was done. The patient died in coma shortly after its completion. Although an autopsy could not be obtained, it was apparent that death had not occurred from hæmorrhage, as the total amount of blood lost, including that retained in the uterine cavity, did not exceed six hundred cubic centimeters. In the circumstances, therefore, it seems justifiable to attribute the death to the pre-existing renal condition, which had become accentuated by the premature separation of the placenta.

These three deaths represent a mortality of 4.68 per cent, but, as has been indicated, only 1.56 per cent could in any way be attributed to the operation. Such results are far superior to those reported by Holland and it might be interesting, but in view of the absence of sufficient data it would be useless, to speculate as to the cause of the deaths in his series. In any event, our figures indicate that those of Holland give a distorted picture of the dangers following supravaginal hysterectomy, and indicate that the results obtained in our hands are essentially as satisfactory as in elective conservative sections done at an appointed time before the onset of

labor or within the few hours following the appearance of uterine contractions. This is particularly emphasized when we consider that in a considerable portion of our cases the condition of the patient when first seen was such as to suggest that infection had already occurred.

In this connection it is interesting to ascertain whether we have unnecessarily sacrificed the uterus and thereby ended the reproductive career of our patients. This can be answered only by studying our cases in somewhat closer detail, and the verdict will depend in great part upon one's point of view. As is evident from Tables 1 and 2, the uterus was removed from eighteen patients solely for the purpose of effecting sterilization. Five of such operations were done at the first and thirteen at a subsequent section.

Whether the removal of the uterus in such cases was indicated depends altogether upon one's point of view; first, concerning the justifiability of sterilization in general, and secondly, upon whether it can best be accomplished by removing the uterus. We have always taken the view that hopelessly deformed or mentally defective patients from the lower classes should be sterilized, and, furthermore, we have made it a rule to sterilize all patients at the third section, considering that in such circumstances the woman had done her duty by society. Accepting such an indication for sterilization, it must be admitted that it can be effected in one of two ways, either by doubly ligating the tubes and burying the proximal ends between the folds of the broad ligaments, or by removing the uterus. If the patient is intelligent, this decision is, in great part, left to her and she is asked whether or not she desires to menstruate after the operation. If she replies in the affirmative, the tubes are ligated, but if in the negative, the uterus is amputated as the easiest and most satisfactory method of attaining the desired end. In addition to these eighteen cases, the uterus was removed from five other women suffering with serious heart disease, as it was felt that in their cases sterilization should follow the first Cæsarean.

Thus we have twenty-three patients in whom the prime or secondary object of the hysterectomy was sterilization, leaving forty-one on whom it was performed for other indications. Eighteen of these were done frankly on account of the patient coming into the service already infected or late in labor. In addition to these, the uterus was removed on account of atresia of the cervix in five instances, neglected transverse presentation in four instances, hour-glass contraction of the uterus in two instances, failure of the bag or bougie to bring about labor in two other instances and dystocia following ventral fixation of the uterus in one instance—a total of thirty-two cases. With the exception of the cases of atresia of the cervix, all of these thirty-two patients were operated upon late in labor and after they had been examined frequently by the vagina, and sometimes sub-

jected to unsuccessful attempts at delivery by other methods. In several of the cases of atresia of the cervix the patient had a high temperature at the time of admission, and it was felt that a satisfactory result could be obtained only by the removal of the organ. Adding these cases to those in which sterilization was the prime indication, we find that they aggregate fifty-five in number, leaving nine others in which the operation was done for some other cause.

The justification for removing the uterus in the former group of cases lies in two considerations; first, the only death which occurred was an operative accident, as compared with the 9.4 and 26.5 per cent mortality following conservative Cæsarean section performed late in labor or after attempts at delivery, as shown by Holland's statistics. Furthermore, as shown in Table 3, sixty of the uteri amputated were subjected to histological study and in twenty-three of them definite histological evidence of ascending infection was found in the shape of leucocytic infiltration or actual inflammatory reaction in the mucosa of the cervix and lower uterine segment. Moreover, in many specimens the presence of bacteria could be demonstrated by suitable bacterial stains. On the other hand, in the twenty-seven patients from whom the uterus was amputated at an appointed time at the end of pregnancy or within six hours after the onset of labor, signs of infection were present in only one, whereas in the twenty-eight patients who were operated upon late in the first stage or during the second stage of labor eighteen presented histological evidence of infection.

TABLE III.
Histological Study of Amputated Uteri.

Duration of Labor	Total Specimens	Inflammatory Changes	Percentage
Before or within 6 h. of Onset.....	27	1	3.7
6 to 18 hours.....	5	4	80.0
Late 1st or 2nd Stage.....	28	18	64.3
Total.....	60	23	38.3

While it is not our intention to contend that all patients with ascending infection would necessarily do badly, it does, however, seem to us that many of them would have done so, and, accordingly, we feel that we have adduced a satisfactory scientific justification for our course of procedure. Moreover, we feel confident that, had the conservative operation been done, or even one of the various types of extra-peritoneal, low cervical or flap operations employed, many more of our patients would have died and we should have had results comparable to those obtained in the first fifty cases of our series, as well as with the figures given by Reynolds,⁴ Routh,² and Holland¹ in late operations.

This being the case, we do not feel that we have unnecessarily sacrificed the uterus, but, on the contrary, we are convinced that our procedure has resulted in saving the

lives of a considerable number of women who would otherwise have died.

It is generally believed that premature rupture of the membranes adds materially to the danger of operation, in that it favors ascending infection. In general this is doubtless true, but our material serves to show that ascending infection may be present without its occurrence, as on examination of the history of the patients, in whose uteri histological evidence of ascending infection was present, it was found that the membranes were intact in nine. On the other hand, in the eleven patients from whom the uterus was removed after labor had been in progress for six hours or more and in which histological examination showed the absence of ascending infection, it was found that the membranes had ruptured in seven and were intact in four instances. While such figures clearly indicate that premature rupture of the membranes does increase the danger of infection, they likewise indicate that the main factor concerned is the actual length of labor, and that whenever it has lasted for more than six hours the probability of ascending infection must always be considered and will increase with every additional hour the labor has lasted.

Furthermore, it is generally admitted that a temperature of 100.4° F. or more during labor raises the presumption of intra-partum infection. Upon studying our material with this in mind, we find that nine of the sixty-four patients presented elevations of the intra-partum temperature. The uterus from five of these presented signs of ascending infection, while in the other four it did not. To put it in another way, it might be stated that, in the twenty-three uteri which presented histological evidence of ascending infection, the condition was preceded by intra-partum fever in only five instances. In other words, the infection was preceded by elevation of temperature only in five out of twenty-three instances. Accordingly, we may conclude that, while the occurrence of intra-partum fever raises the presumption of ascending infection, its absence by no means indicates that it has not already occurred. Here again we must draw a conclusion similar to that in connection with premature rupture of the membranes; namely, that the great danger arises from the prolongation of labor rather than from any of the factors upon whose occurrence we have in the past laid such weight.

For these reasons we have come to regard the late Cæsarean as an extraordinarily dangerous operation and, in such cases, if we are not prepared to sacrifice the uterus, we must resort to pubiotomy or the destruction of the child if we wish to safeguard the patient, whereas, if we resort to radical measures, we may obtain results comparable to those following conservative Cæsarean section done at an elective time either before or just after the onset of labor.

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AIR EMBOLISM FOLLOWING VARIOUS DIAGNOSTIC OR THERAPEUTIC PROCEDURES IN DISEASES OF THE PLEURA AND THE LUNG

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Probably it would not occur to any physician to suggest that air emboli following such common technical procedures as puncture to clear up the nature of an effusion in the thoracic cavity, or irrigation of a long-standing empyema cavity with an antiseptic solution such as bichloride of mercury, hydrogen peroxide, or Dakin's solution, or change of position of the tube draining such a cavity, may cause complications that could endanger the life of the patient. Roger,¹ a French physician, in 1864 first called attention to these accidents in his observation on a child eight and a half years of age suffering from a chronic empyema cavity. While the customary irrigation of this cavity with an antiseptic solution, with the use, perhaps, of a flow stronger than during the other irrigations, was being performed, the child fell backward from her sitting position, unconscious. She showed clonic contractions of both upper and lower extremities, similar to those of epilepsy. This attack lasted one hour. The child lay drowsy for the rest of the day. By the next day all the symptoms were gone, and she felt normal. Basing their opinion on this incident and others similar to it, Roger, and later on other French physicians, Besnier,² Legroux,³ and Lépine,⁴ concluded that these attacks were of nervous origin—an irritation of sensitive nerve fibres in the pleura, which transmitted this irritation to the central nervous system. They spoke of this reaction as a *pleural eclampsia* or *pleural epilepsy*.

These first clinical experiences were gained from accidents following irrigation of an old empyema cavity, as in Roger's case, or from diagnostic puncture of the chest wall for a supposed circumscribed abscess formation. For a long time irrigation was carried out without any complications. Then, in one case the patient fell unconscious; in many instances there was an aura at the onset. Sometimes the patient complained of visual disturbances, and at the same time had a prickling pain in the discharging side. Headache and dizziness at times accompanied the onset. These initial signs were followed

by clonic or tonic contractions of one limb; or all the limbs were involved. The two forms sometimes alternated. Even the facial nerve was affected. These motor troubles were accompanied by eye symptoms,—dilated pupils on the affected side, on the opposite side, or on both sides. Respiration ceased or became of the Cheyne-Stokes type. At the same time there was also abnormality of the heart action, the pulse becoming accelerated and very thready. At this stage the unconscious patient often had involuntary movements of the bowels. If a sudden change in the clinical picture did not take place, death terminated these so-called pleural eclampsies. At autopsy there were very often no positive findings.

In cases where the patient recovered after several hours or even after some days, a paralysis very often persisted for a time, generally in the muscles which had been most affected by the motor disturbances. St. Philippe⁵ observed a left-sided palsy in a case of left-sided empyema. No general rule can be made out about the relationship. In Walcher's⁶ case the paralysis lasted for two days. The average duration was from a fraction of an hour to several hours. Aphasia of short duration was an exceptional symptom after these pleuro-pulmonary complications. Blindness was the most impressive sign noticed by the patient at the onset of the attack. It lasted for some hours or for days. During an attack the pupils were generally dilated, not reacting, and often unequal. Marble-like discoloration of circumscribed spots of the skin, as the forearm, or calf, was noticed in a few cases. The great variety in the clinical symptoms in these attacks is striking. The negative findings at autopsy strengthened Roger's opinion as to the nervous etiology of these complications.

Raynaud⁷ in 1875 also supported this belief in the origin of these pleural reflexes, as did Bouveret,⁸ Brouardel,⁹ de Cérenville,¹⁰ Desplats,¹¹ Dumontpallier,¹² Gilbert,¹³ and Letulle.¹⁴ Cordier¹⁵ and Gilbert tried to verify their opinion by experiments on animals. In Da Costa's Modern Surgery¹⁶ Dr. Toland is quoted as hav-

ing observed four cases of pleuritic epilepsy following irrigation with Dakin's fluid. Da Costa refers to Quénu as having seen intense dyspnoea, great shock, disturbances of respiration and circulation, convulsions, and death follow injections of the blandest fluid (de Cérenville: pleural epilepsy).

Similar accidents were observed after the performance of other therapeutic procedures, such as introduction of a probe or a rubber catheter into a narrow sinus of an old empyema cavity. In cases in which the canal had to be dilated by a laminaria stick, these reflexes also occurred. It is interesting to note that in all these cases with complications there was bleeding from the cavity, showing that a mechanical lesion of the fibrous wall of the canal or of its granulating surface had occurred.

Brandes¹⁷ published the report of a case in which he tried to outline the extent of an empyema cavity by an injection of bismuth paste through the sinus. The rubber catheter apparently injured the wall of the cavity. Most of the bismuth escaped alongside of the catheter. The patient had a spasm, with deviation of the eyes, and died after 20 hours. At autopsy, bismuth was found in the smallest blood vessels of the cerebral cortex of both hemispheres. On examination of serial sections from the wall of the sinus, the bismuth could be traced up to the pulmonary capillaries and veins. The catheter had injured the granulations and opened some venules underneath. Bismuth was also found in other capillaries of the greater circulation.

Puncture of the pleural cavity is quite a common procedure, familiar to every practitioner. For example, in the course of a pneumonia we suspect an exudate in the affected pleural cavity, and perform a puncture to clear up the diagnosis. Or the clinical findings and the X-rays make it very likely that we have to do with an interlobar empyema or a lung abscess; the diagnosis is made certain by the puncture. A clinical picture similar to that given above may be encountered when carrying out any of these procedures. Two striking cases may illustrate the truth of this statement.

Jessen¹⁸ was giving local anaesthesia to a patient thirty years of age with tuberculosis of the lung for a thoracoplasty over a tuberculous cavity, and was trying to make a special deposit of the fluid on the pleural side of the rib. At this moment the patient had an attack of coughing, and fell unconscious, with partial pallor, cyanosis, and clonic twitchings of the left arm. Blood-stained sputum was observed. Respiration and heart action were at first normal. Later on the reflexes were found to be absent. The pupils became narrow, and did not react. The patient died 16 hours after the onset of the attack. The autopsy showed a puncture wound in the indurated wall of the tuberculous cavity. The mucus

in the bronchi was stained with blood. No air could be found in the vessels of the brain.

A second case, observed in Professor Clairmont's clinic (Zürich), was published by me in the *Deutsche Zeitschrift für Chirurgie*.¹⁹ A street car conductor, thirty-four years old, had suffered with a recurrence of bronchitis which had developed into broncho-pneumonia. On the supposition of the existence of a circumscribed abscess, the right lung was punctured twice without result and without accident. Relying on the typical clinical picture, the operator repeated the puncture in several places some days later with the same negative result. A week later a new attempt to help the patient was made. The X-rays showed in the right lung a circumscribed area filled with fluid. Resection of a segment of the fourth rib was made under local anaesthesia. Before going further a puncture was made and some bloody fluid was withdrawn into the syringe. For examination of this fluid the syringe was detached, the lumen of the needle being now in free communication with the atmosphere. At the moment the syringe was taken off the patient collapsed. He became unconscious, with fixed pupils, and clonic convulsions (twitchings) of all muscles. Some minutes later he became conscious again. Within two and a half hours he had gradually recovered completely, except for loss of sight. The ophthalmoscopic examination revealed a normal fundus. The blindness lasted for three days. The patient had an increased muco-purulent expectoration. The signs of abscess cleared up without further operation. The patient improved every week, becoming finally normal after six months, when he resumed his occupation.

In these two cases the presence of air embolism seems very probable, but the diagnosis was not confirmed either by the clinical findings (ophthalmoscopic examination) or by autopsy.

The first clinical observations of what proved to be experimental air emboli, unintentionally produced, were made in cases of accident in pneumothoraxtherapy for tuberculosis. The phthiseotherapists frequently employ artificial pneumothorax in cases of unilateral pulmonary tuberculosis. It is especially to Forlanini²⁰ (1882) that we owe the introduction of this procedure in medicine. He advised the so-called puncture method, in which the needle is introduced directly through the thoracic wall. Accidental puncturing of the lung may occur, and with this there may be trauma of some engorged veins in a tuberculous area underneath the surface of the lung, with sequele, which we shall consider later. This grave danger, with subsequent air emboli, led J. B. Murphy²¹ to suggest exposure by a preliminary incision of the parietal layer of the pleura, with introduction of the needle into the fissure of the pleural cavity. Brauer²² became a strong supporter of Murphy. He was the first to recognize, in 1912, the danger of air embolism in thoracic work,

and rejected the opinion held by Roger and others as to these so-called pleural reflexes. Wever's²³ experiments are convincing. Forlanini did not admit the possibility of the presence of air emboli. He maintained the old conception of the nervous origin of the convulsions.

Among the phthiseotherapists using pneumothorax-therapy (Spengler,²⁴ Saugmann,²⁵ Giesemann,²⁶ Sundberg,²⁷ and v. Voornveld²⁸) air embolism as the cause of the fatal outcome during this treatment is generally accepted. Jessen believes that the frequency with which air embolism occurs is much greater than is recognized, and in support of his view is the statement of a colleague of Dr. von Voornveld, that he had given up this therapy because he had had three deaths in 12 cases. Many of the cases are registered as death from chronic myocarditis, cachexia or shock. This evidence shows that prominent physicians in Europe do not agree with the conception about pneumothorax-therapy of Da Costa, who points out that there is little danger in the method, although possibly trouble may follow. That statement may perhaps hold good when the Murphy-Brauer method with the incision is employed. But in Europe, where the Forlanini-Saugmann puncture technic is generally used, there is some danger that this method, even in the hands of an experienced physician, may cause air emboli, although the technic has been worked out to diminish to a great extent the possibility of danger. The first puncture presents the greatest risk, but in cases of a partial pneumothorax the danger is always present. When the operator is refilling or increasing the air content, the needle may either pass into a strand of adhesion, thereby opening a vein, or may pierce infiltrated lung tissue. The same complication occurs when the strand of adhesion breaks through near its attachment to the lung surface. It is on account of this possibility that the adhesions are cut through near their attachment to the pleura when Jacobaeus'²⁹ thoracoscopy is employed. Even then the danger is not completely eliminated.

The following figures will give some idea of the frequency with which these complications may occur in the hands of even expert operators. Forlanini³⁰ had, in 10,000 punctures on 134 patients, 12 accidents, 5 with temporarily serious conditions, but all with final recoveries. In a second report he had in 1454 insufflations on 28 patients 4 complications without a death. Saugmann³¹ records 970 operations on 54 patients without complications. In a second series³² he had in 215 first punctures and 5500 follow-up treatments 2 cases of embolus. Extracts from some histories may illustrate these accidents.

Saugmann³³ tried to introduce air into the chest of a man twenty-two years of age, with unilateral pulmonary tuberculosis, which had existed for some five years. A first session was without result. A wide manometric excursion, corresponding to inspiration and expiration—

proof that the needle is in the pleural cavity—was not shown. At a second attempt the oscillations of the manometer were only minimal, and the procedure was given up. Later, a third effort was made with the same result. The patient was then told to take a deep breath. At this moment he turned pale, and complained of dizziness. The needle was taken out. The patient became unconscious, and vomited, and the breathing became very irregular. On the right side the pupil was large; the eyes showed divergent strabismus. The pulse could not be felt. Although various stimulants were employed, the breathing stopped also. The autopsy revealed two prick lesions in caseous pneumonic lung tissue, with some suffusion of blood. No large blood vessel was implicated.

In a second case of Saugmann,³⁴ a woman, aged thirty-two, with a long-standing left-sided tuberculosis, the patient was very nervous. The inserted needle was connected with the manometer, but the latter did not show any wide excursions. An obstruction in the canal of the needle was supposed to be the cause and a stylet was passed. The patient took a deep breath. The stylet, on being withdrawn, showed some blood. At this moment the patient said that she felt giddy. The needle was suddenly withdrawn. The patient became pale. She lay on her back, unconscious, the eyes and the head turned to the left, with the pupils at first contracted, later dilated. Respiration and the heart's action became irregular; the pulse stopped and death occurred. At autopsy a mechanical lesion made by the needle in the lung tissue was found, with bloody suffusion of the whole area. On microscopic examination the damaged vein could be seen. In the vessels at the base of the brain, especially in the Sylvian artery, gas bubbles were found.

Spengler³⁵ cites the case of a girl, eighteen years of age, with left pulmonary tuberculosis. Pneumothorax-therapy was being carried on with good results. At one session, when the operator was trying to increase the air content of the cavity, the manometer showed by its wide oscillations that the needle was in the right place. Air to the amount of 250 c.c. was injected. The patient became agitated, made a quick movement with her left arm, and suddenly fell unconscious. The needle was taken out immediately. The pulse could not be felt. Pale, with dilated pupils, the patient was breathing quite superficially. A circumscribed marble-like area on her left forearm was noted. Clonic contractions of all the muscles were followed by tonic cramps in the right upper arm. When she groaned, a palsy of the right facial nerve could be seen. For several hours she slept. Twitchings recurred several times, especially on the right side. She had an involuntary bowel movement. Death occurred after 3 days. Autopsy was refused.

Carpi,³⁶ when beginning the Forlanini technic on a patient who had been sick for 3 years, felt sure that the needle was in the pleural cavity. He started with 10 c.c.

of nitrogen. At this moment the patient complained of pains in the region of the puncture. She had a sensation of pressure, became unconscious, and had clonic convulsions on the right side, which later implicated the left side also. Trismus and opisthotonus were caused by these contractions. The respirations became frequent and superficial, with a pulse of 140. For a short time the patient was cyanotic, and sweated profusely. The pupils reacted to light. This attack lasted only a few minutes. After a short interval a second similar one followed. She made an uneventful recovery.

Before passing to the study of the etiology of these complications we shall consider the other procedures in which these serious accidents may occur.

Decortication (Delorme, Fowler) is advisable in cases of old empyema with a persistent sinus, to relieve the patient from the annoyance of a constant discharge of pus. Delorme's³⁷ procedure tries to avoid the great disfigurement of the Schede thoracoplasty by including the dense fibrous thickened visceral layer of the pleura of the lung, so that the lung can expand to its primary volume. This thick membrane holds the lung down in its contracted state. Fowler,³⁸ of Brooklyn, used the same technic at the same time without knowing of Delorme's procedure. A modification of this operation is Ransohoff's³⁹ dissection of the pulmonary pleura for cases in which it is impossible to liberate the lung from its encircling coat. Parallel incisions are made a quarter of an inch apart, crossed by a second series perpendicular to the first. By this procedure expansion of the lung is probably rendered more gradual.

In these decortications many blood vessels, especially veins, in the superficial part of the lung are mechanically injured, the injuries being analogous to those that may be caused when irrigating this wall with a strong flow, or when introducing a tube into this cavity, which ploughs a groove on the surface of the lung (Brandes' observation). Confronted in all these cases with similar pathological changes of the pleura and neighboring lung tissue, we encounter a similar danger of air emboli (Weill).⁴⁰ In pneumothoraxtherapy we have an analogous process when in a partial pneumothorax one tries to break up some adhesions. These adhesions may become loose, and leave a defect in the superficial layer of the lung. The lumen of an opened vein may be kept open by its position in fibrous, dense tissue, so that the air comes into direct contact with the venous circulation. To diminish this danger Jacobaeus,⁴¹ in 1913, devised an endoscope for the thorax, through which he burns off the adhesive strands along the costal pleura (thoracoscopy).

Careful precautions have to be taken in mobilizing the costal pleura, especially at the apex of the lung, when an attempt is made to insert a fat or a paraffin plug (Tuffier,⁴² Baer⁴³). The veins of these thickened pleurae communicate with the veins in the adjacent lobe of the

lung. The same danger is encountered in mobilizing the costal pleura for collapsing an empyema cavity, previously sterilized with Dakin irrigation.

In all the previously described operations the procedures were done near the chest wall. In pneumotomy and pneumectomy the operator comes nearer to the vessels at the hilus, with the larger veins under negative pressure, at least during inspiration. In these cases the possibility of complications from air embolism is always imminent, more so when sharp dissection is employed than when one comes up behind the necrotic wall with the Paquelin cautery or with a blunt instrument, such as the finger.

Passing through lung tissue with the cautery in attempting to reach a bronchiectatic abscess in a patient thirty-one years old, Quincke⁴⁴ was stopped by profuse bleeding. By means of gauze tampons the bleeding was controlled. The patient complained of a prickling sensation in both arms, and dizziness. He looked pale and collapsed with irregular, feeble pulse. The respirations became less frequent, and stopped. Fifteen minutes after the onset the patient was dead. At autopsy an open vein was found next to a bronchus which had been burned off by the cautery. The neighboring lung tissue was carbonized.

Wever⁴⁵ cites a similar case of a girl, nineteen years of age, with bronchiectasis. The intention was to open the cavities in several stages. During one of the procedures the patient coughed and at the same time profuse bleeding occurred in the wound, which was stopped by gauze packing. The patient vomited and had tonic cramps in both arms. She was unconscious, and turned her eyeballs upward, with widely dilated reacting pupils. Marble-like patches, with a distinct outline, on the body and the extremities indicated definite vascular disturbance. The speech was dysarthric. A left-sided palsy of the facial nerve was noticed. For 24 hours she remained about the same, after which she gradually recovered.

To clear up the etiology of these complications we must consider the conditions in the normal pleura so far as they concern its sensibility. From a series of experiments which I began in the surgical clinic in Zürich, Switzerland (Professor Clairmont), and which are being continued in the Surgical Hunterian Laboratory (Professor Halsted) of the Johns Hopkins University, I have come to the following conclusions:

From irritation of the normal pleura of different animals, mechanical, chemical, or by the electric current, we do not get a constant specific reaction by way of the central nervous system, as we should expect if the so-called pleural reflexes were the real source of the above-mentioned complications in the different operations upon the chest. In the costal pleura we have sensory nerve fibres (intercostals), mechanical or chemical, irritation of which causes general protective movements, similar

to those which we see in other parts of the body (self-protection).

In smaller animals (rats, guinea-pigs, rabbits) faradic stimulation of the parietal pleura causes clonic contractions of the upper extremity of the same side. In dogs I have obtained only local contraction of the thoracic musculature, with no movement in the adjoining extremity. Faradic stimulation of the pulmonary or mediastinal pleura produces no muscular contraction at all in any of these animals. Stimulation of the diaphragmatic pleura with the faradic current gives only contraction of the diaphragm. This fact seems to indicate that the diaphragm gets its sensory nerve supply from the phrenic nerve only and not from the neighboring intercostals also. In dogs, as was stated above, even by irritating an area of the diaphragm next to the thoracic wall one gets only a contraction of the diaphragmatic muscle. No contraction is seen in the neighboring intercostal musculature. With faradic stimulation of the parietal pleura adjacent to the diaphragm in smaller animals, contractions are observed, in addition to those in the chest muscles, in the upper and lower extremity of the same side. In dogs only local muscular contractions are seen.

The vagus (pneumogastric) fibres running with the bronchi and all the branches of the bronchial tree carry pain sensation centripetally, causing protective movements in the upper extremity on the same side in small animals. After chemical irritation (with iodine) of the pleural cavity, the substance spreads through the pulmonary pleura into the lung. Resection of the vagus nerve in the neck inhibits these contractions. Stimulation or paralysis of the sympathetic nerve has no influence on these protective movements.

In all the cases of empyema in which these complications have been observed we have to deal with a long-standing condition. The pleura is thickened and covered with a granulating surface. The sensory nerve fibres in the area of the empyema cavity on the side of the thoracic wall are strangulated by mechanical pressure exerted by the encircling fibrous tissue. They are not functioning, and any reflex movements along these fibres are made impossible. Moreover, the lung tissue adjoining such a constantly discharging chronic infected cavity is altered and has become fibrous, as a consequence of reaction against the infection. In the majority of the cases of empyema, perhaps in all (C. Beck),⁴⁶ the primary focus of infection is in the superficial layers of the lung; the pleural cavity becomes affected secondarily. The fibrous tissue formation around an infected area represents a natural reaction of the body to combat the invading microorganisms. Around every lung abscess we have transformed tissue, partly infiltrated, partly fibrous, with the resultant changes around the blood vessels,

particularly the veins with their weaker walls. The tissues about a bronchiectasis show similar changes.

In an empyema cavity the only sensitive elements are the sympathetic nerves, running with the blood vessels into fibrinous deposits which outline the wall. In the lung we have the vagus fibres along the bronchioli.

These are the pathological conditions which are found in variable degree and extent in all cases in which we are threatened with an air embolus. In tuberculosis of the lung we find similar changes taking place. But in that disease, in cases suitable for pneumothoraxtherapy, we do not find such uniformity of arrangement of the indurated tissue. Places showing little change are close to fibrous areas. In the center of the pathologically indurated areas we may have a cavity, or at least a circumscribed necrosis (caseous pneumonia). In these lungs we find all the different stages of the fight of nature against the invader. In all cases of air embolism the pneumothorax needle always passes into an area showing a specific process of natural repair. The pleura in these cases may be still very sensitive, if only relatively small parts of the lung are involved by the tuberculous disease.

In the wall of an empyema cavity with its underlying, chronically inflamed lung tissue, in the neighborhood of every active infection in the lung, the blood vessels are involved in dense fibrous tissue formation. In an earlier stage, when the infection is still active, the tissue shows a certain amount of infiltration. The vessels are suspended between the fibrous strands. There is a constant pull on these vessel walls when these fibres contract, especially on the veins. When their walls are mechanically injured, the veins cannot contract to the full extent, being hampered by their attachments in this inelastic tissue. The musculature of the vein, being weaker than that of the artery, is insufficient; the opening in the wall of the vein persists; from the hollow of the needle, or from the neighboring cavity (pleural cavity, bronchus) air or gas enters; and we have an air embolism. The result following any injury of the vessel wall shows the inability of these veins to contract to the same extent as they would do in normal tissue.

The blood in the lung gets its impulse from the right ventricle through the pulmonary artery. The sucking effect of the left atrium and the larger veins of the lungs is not so efficacious. The combined force of these two factors, together with the act of respiration, makes the current continuous. The sucking influence from the larger veins has a minor effect, in proportion as the vein is situated in the superficial parts of the lung, away from the hilus. A lesion of such a vein by an instrument allows the air, remaining under atmospheric pressure, (cavity, bronchus), or gas from a pneumothorax, to enter through the opening. The friction exerted by the air upon the vessel wall causes by reflex action a contraction of the

muscles innervated by the sympathetic nerves. This contraction is made ineffective by the attachment of the wall of the vein to the encircling fibrous tissue. At the same time this friction of air and blood along the wall stops the blood proximal to the air. This friction and the internal resistance of the air stops the movement in the blood column proximal to the lesion. No further movement is possible, unless the sucking action from a larger branch of a pulmonary vein comes to their assistance. The air embolism does not become clinically evident. This is the reason why this complication is not reported very often. In the different operations upon the chest wall a lesion of the veins happens quite frequently, but it does not make itself evident.

The force has to come from a larger vein under negative pressure in the neighborhood of the wounded vein in order to bring the air and the blood by aspiration into the left atrium. In several experiments on dogs the pressure in the pulmonary vein next to the hilus was tested with a manometer through an intercostal opening in the thoracic wall. The pressure was found to be -0.3 to -0.4 cm. water; i.e., negative. From the left ventricle the air can be dispersed all over the body. The air embolism renders itself manifest especially by the trophic changes in the brain and in the eye-grounds (retina). In the larger vessels the air causes a contraction of the wall; hence the smaller vessel may be closed at its junction with the larger vessel.

The distance between the point of the mechanical lesion of the vein wall and the junction of this vessel with a larger vein under negative pressure, whence the blood in the lacerated blood vessel, together with the air that has entered, can be aspirated, is the essential point in inaugurating the onset of a clinically manifest air embolism.

In cases of unilateral tuberculosis of the lung treated by artificial pneumothorax the injured vein may become connected only with the air in the needle. Then we have the conditions described above. But when the accident happens when the gas is flowing out from the apparatus under pressure, the air embolism is much more dangerous. These are the cases ending in sudden death, in which at autopsy we find a great number of gas bubbles in all the smaller vessels of the brain.

The mechanism of air embolism in an empyema cavity, caused by a change of a tube, the use of a probe, dilatation with a laminaria stick, irrigation, or injection, is well illustrated by Brandes' case, in which bismuth paste was found filling up the vein from the point of injury to a larger branch, with emboli in the capillaries all over the body, especially in the brain and in the intestine.

Mechanical injury of the blood vessel is facilitated in all abscess cavities by partial necrosis of the wall, caused by the continuous effect of the presence of pus. The blood vessels resist this process for a longer time, and

therefore become exposed. Under these conditions a strong current of fluid may open a vein. A proof of this statement is that in the history of these cases we find that the fluid was observed coming back mixed with blood.

The recorded cases of air emboli in thoracoplasty have the same physiological and pathological underlying causes as empyemata. The knife or the blunt dissecting finger is always the primary faulty agent. To diminish this danger the use of the cautery was introduced, particularly for operations in the deeper part of the lung (bronchiectasis, pneumotomy). A path to the abscess cavity is made through the lung tissue by burning which closes all vessels by a thick escharotic membrane. Near the hilus even the Paquelin cautery cannot close the pulmonary veins tight, as was shown by the cases referred to above. By using the finger as a gently advancing dissector (Lilienthal)⁴⁷ this danger may be somewhat diminished. Moreover, we avoid the danger of a secondary hemorrhage when the eschars become loose in these infected wounds.

Complications showing a similar clinical picture may occur when we are performing the operations on the chest cited above, in which another cause for the complications is present. Without a thorough knowledge of the clinical features of air embolism, the physician can never be sure of the real source of these accidents. After all, a number of cases on the border line will remain undetermined.

We are constantly having to deal with patients who have lost weight and strength during a long illness. Complications from various sources arise much more easily in these patients, as they have no resistance. The quick release of a large amount of fluid out of a cavity in the chest can produce a collapse, caused by a temporary anæmia of the brain (empyema, ecchinococcus). In conditions with chronic inflammation of the lung, such as empyema, some veins may be blocked by thrombi (v. Dusch).⁴⁸ If they are mobilized during a therapeutic procedure, these clots follow the same course as the air, and we have a very similar clinical picture. Vallin,⁴⁹ in 1875, was the first to announce the opinion that these epileptiform attacks may be caused by capillary emboli from thrombi in pulmonary veins. Thrombi in the pelvic plexus or in the lower extremities may be mobilized during an operation. The pulmonary embolus gives a picture similar to that produced by a heavy air embolus. Autopsy will clear up the etiology. Chronic myocardial lesions may cause a collapse during an operation. The air embolus can be ruled out only by thorough examination. In a similar way insufficiency of the adrenals may become evident by a collapse with death. In only a few cases are the unimportance of the clinical signs and the short duration striking. The past history of these patients reveals a neurotic constitution. No

local signs of cerebral affection are seen. These conditions have a neuro-hysterical basis and are easily recognized.

The features of the clinical picture of air embolism following the different procedures mentioned above are so varied that it is very difficult to include them in a definite classification. Unfortunately, many of the histories of the reported cases do not mention several clinical features which we would expect in air emboli, and which would be of great importance in the differential diagnosis of these complications. More thorough examination of these conditions is necessary in order to furnish wider experience in such cases.

Pallor of the patient is often the first sign of imminent danger. Circumscribed patches of cyanosis on the skin present a marble-like appearance, a further proof of disturbances in the circulation. The patient complains of a sudden pain in the chest on the affected side. He cries out, saying that he feels giddy or ill and that he has black spots before the eyes. In many instances he becomes totally blind. Irritative pains in the injured side of the chest and severe headache aggravate the condition. The patient swoons, very often with no preceding aura. Unconsciousness comes on suddenly. The pulse is altered, perhaps cannot be felt any longer. Dyspnea passes into stertor; the breathing becomes gasping; respiration stops. Involuntary evacuations precede the sudden death. In other cases pulse and respiration become very frequent. If the patient is still conscious, he complains of oppression in the chest. Later on the pulse becomes feeble. Respiration is of the Cheyne-Stokes type. The pupils are generally widely dilated, and do not react. In a few reported instances they were different on the two sides, perhaps with strabismus of the convergent or divergent type. If death occurs shortly after the onset of the complication, an autopsy should be done as soon as possible after death, to eliminate any post-mortem changes in the brain, the organ of greatest importance for the diagnosis.

After having passed through the stage of these preliminary symptoms, the attack may come to an end, the patient recovering gradually from his comatose condition.

In the majority of all cases clonic or tonic convulsions give evidence of an excitation of the motor areas, beginning in the eye muscles or in the upper extremity, and very often becoming generalized all over the body. Spasm of the back muscles gives rise to opisthotonus. From the histories of the reported cases it is impossible to trace a definite relationship between the affected side of the chest and the location of the most violent convulsions. In the literature we find the greatest variation in this feature. These contractions are followed by a paralysis, which varies in location and duration. Usually it lasts only a short time, from some minutes to several

hours or several days. The palsy is generally most marked in the limb that is most affected by the convulsions, and remains there longer than in the other regions, perhaps even for months. The eye-sight is altered in an analogous manner, its return to normal being governed by a similar law. The disturbances in the sensory-motor region are shown also by circumscribed areas of hyperaesthesia and anaesthesia, and by different forms of paræsthesia.

Examination of these cases of air embolism discloses the fact that the clinical findings are quite variable. From a study of the pathology and physiology of these lesions we must try to trace the effect of the temporary embolism in different organs, the presence of which was indicated by the clinical pictures just described.

The ophthalmoscopic examination during an attack and later on at periodic intervals is of the greatest importance. The findings in the reported cases are divergent. Raynaud found in his case a serous suffusion around the papilla and a venous hyperemia. Schnitzler⁵⁰ noticed in de Cérenville's case a venous stasis combined with capillary hemorrhages. In the observation by Clairmont, which was published by me, no abnormality could be found immediately after the accident or later. In a case of air embolism following pneumotomy for gangrene of the lung, Becker⁵¹ saw the air passing through the arteries of the retina in the form of gas bubbles. Later on, when the air was passing through the capillaries, strands in the form of fine white silver glittering lines filled the fundus. Subsequently the veins became enlarged. In the middle of the veins a white strand was seen (Stargardt).⁵² The air seemed to be carried in the center of the vessel. After a short time the fundus looked normal again. Even after weeks changes caused by temporary thrombosis of the smaller arteries and capillaries can be seen. In cases with negative findings of the eye grounds the source of embolism may lie in the occipital lobe.

The clonic and tonic convulsions with subsequent palsy are due to nutritive changes in the motor area of the cortex. Partial temporary thrombosis in the medulla oblongata is followed by sudden death due to blocking of the vital regulative centers of respiration. The microscopic examination of the brain in these cases will show the cause of death. Spielmeyer⁵³ found liquefaction of the cells of the cortex as a typical condition. He noticed also an incrustation around the nerve cells of the nerve plexus. The glia reaction was negative. In cases in which death had occurred after several days, Spielmeyer found marked proliferation of the glia cells in circumscribed areas.

Air blocking the smaller blood vessels in the intestine (stomach, duodenum) causes a typical ulcer formation in the mucous membrane. This was found by Keller⁵⁴ in several cases at autopsy. When the patient has not partaken of meat before the operation which was com-

plicated by air embolism, a careful examination of the faeces during the following days will reveal traces of blood. Of course when the past history and the examination are suggestive of ulcer, or of tuberculosis of the intestine, this finding is of no use for the diagnosis of embolism. Albumin in the urine, and epithelial casts,—if they were not present before the accident—are quite significant of temporary nutritive blocking of the renal system.

Besides these different clinical findings the evidence of injury of a blood vessel during the diagnostic or therapeutic procedure is of the greatest value. Blood on the stylet after puncture is very suggestive of a lesion. The patient may have at the same time an attack of coughing, and spit up mucus mixed with blood. Bleeding during irrigation of an empyema cavity or during the changing of a tube in such a cavity is a sign to be noted as one of imminent danger. In thoracoplasty and in pneumotomy sudden bleeding is also of great diagnostic value. In all accidents accompanied by the symptoms described above we must think first of an air embolus, unless we can rule it out by the lightness of the attack in a patient who has a nervous make-up (nervousness, neurasthenia, epilepsy) and is weak from a long illness.

The *prognosis* in any case of air embolism after an operation on the chest depends upon the amount of air entering the blood stream, and also upon the region in the brain which is most seriously involved. The extension of the condition, especially to vital centers, causes sudden death. The clinical signs during the first attack give a fairly practical indication of the extent of the lesion. If the patient survives the first attack, the outlook is not bad, and he will gradually within a short time recover from all the bad effects of the accident. The time necessary for convalescence is proportional to the seriousness of the clinical picture at the onset. In the great majority of cases all the symptoms are temporary; as time goes on, they disappear. The air passes through the capillary system, causing a temporary blocking of certain areas in the brain, with concomitant nutritive changes. The heart action, which regulates the blood current, is very important in forcing the air through the capillaries, in this way diminishing the duration of the thrombosis in essential parts of the brain.

These points afford a sound basis for our therapeutic measures to combat the effects of these complications. The procedure that has been the source of the accident, must be stopped at once, thus removing the causative factor. The needle used in the puncture or injection or in the pneumothorax treatment is taken out. The irrigation in a case of chronic empyema is stopped. The tube or the probe causing the injury is removed. In pneumotomies where profuse bleeding shows that the vessel lesion is in the deeper part of the lung, packing the whole cavity with gauze will prevent the harmful air

embolus. For these cases more particularly the use of a positive-pressure apparatus, to diminish the negative pressure in the larger pulmonary veins, recommended by Tiegel,⁵⁶ has proved to be very satisfactory.

When confronted by an air embolus the physician concentrates his attention on strengthening the heart and increasing the blood current through the brain, in order to force the air from the capillaries as soon as possible and to drive it to the lung, whence it can be expelled from the circulation through the alveoli. Intravenous injection of heart stimulants helps to strengthen the action of the heart muscle. An intravenous injection of adrenalin is followed by a contraction of the vessels of the splanchnic area (intestines) and the skin. This is a suitable and helpful procedure to improve the blood supply of the brain. The head should be put low, to increase the effect of this procedure. Jessen⁵⁷ has reported good results from a venesection.

If the breathing stops, rhythmic traction of the tongue and faradisation of the phrenic nerve should be employed. Artificial respiration is contraindicated, because deep inspiration might enlarge the opening in the vein and give rise to a new embolus. Morphine should never be used because of its paralyzing effect on the respiratory center.

As a useful prophylactic procedure, when doing these operations on the chest, bend the head of the patient to one side so that it is not the highest point of the body. With the head in this position, in case of air embolism, the air bubbles do not have the tendency to reach the capillaries of the brain. This was proved by experiments which I made on middle-sized dogs in the Surgical Hunterian Laboratory of Professor W. S. Halsted. The dog was put on his right side, flat. Under ether anaesthesia an opening was made in the left chest wall, in the fifth intercostal space. An injection of 2 c.c. of air was made into the pulmonary vein at the hilus. On ophthalmoscopic examination no air could be seen. A second injection of 2 c.c. of air was made. No air was seen passing through the blood vessels of the retina, but a gurgling sound was heard on squeezing the heart (left atrium). As soon as the dog was put in a semi-erect position, air bubbles appeared in the eye-ground. The dog died without any convulsions. This experiment was repeated with the same findings.

SUMMARY

1. Following various diagnostic and therapeutic procedures on the chest (puncture, irrigation of a cavity, changing tubes, introducing a probe or a rubber catheter, bismuth paste filling, decortication, thoracoscopy, fat or paraffin plompage, pneumotomy, pneumectomy), complications may ensue which have been proved experimentally and clinically to be caused by air emboli.

2. The sudden release of a great amount of fluid in emaciated people with a neurotic constitution (neurasthenia, epilepsy) may cause a similar clinical picture. Because of its slowness and short duration, with lack of localized cerebral symptoms and serious sequelæ, this condition is considered to be due to shock.

Chronic myocardial lesions or an insufficiency of the adrenals may cause sudden death during the performance of one of these operations on the chest wall.

A similar clinical picture is seen in embolism of the pulmonary artery. Death, accompanied by these signs, may occur also in a few cases of emboli in the brain following thrombosis of pulmonary veins. The autopsy will clear up the diagnosis.

3. It was demonstrated by experiments that we do not have a pleural reflex, even in the normal pleura, which would explain these complications. They are proved by the accidents in pneumothoraxtherapy to be due to air emboli.

4. The pathological condition of the lung and the pleural sheaths is the same in all these cases. The lung tissue shows, in a circumscribed area, a condition which is partly an infiltration, partly an induration of the tissue, where the blood vessels, especially the veins with their weaker walls, are fixed in a distended position. When mechanically injured, they cannot, with these rigid surroundings, collapse as usual; the hole stays open. The distance of this wounded vein from a branch of a larger pulmonary vein under negative pressure, which can aspirate the air with the stagnating blood, decides whether the air embolism will become evident. Absence of this condition is the reason for the infrequency of air emboli.

5. The clinical pictures of these air emboli show a wide variety. They should be made more exact by further thorough study of these complications by means of ophthalmoscopic, stool, and urine examinations.

6. The best therapy is the preventive one. When an air embolus occurs, stop the operation immediately. Put the head of the patient low. Reinforce the heart action with stimulants. Adrenalin injections, administered intravenously, will increase the amount of blood passing through the brain by diminishing the blood supply in the splanchnic areas (intestines, skin).

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THE EFFECT OF SALINE PURGATIVES ON THE ABSORPTION OF OTHER DRUGS*

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INTRODUCTORY

The present investigation was begun as the result of a personal experience. While suffering from an attack of the gripe one of the writers took for the relief of a severe headache a full dose of aspirin which had generally proved efficient for the purpose on other occasions. In the present instance no relief was experienced. The patient then swallowed a tablet of phenacetin and salol, also without relief. This was followed by five grains of pyramidon and this also did not prove any more efficient in the relief of the headache and pains than the other antipyretics. On pondering over the cause of the ineffectiveness of the above medication it was recalled that, before having recourse to the antipyretics, the patient had taken a saline purgative. It therefor occurred to him that possibly the saline purgative might have played a rôle in preventing or retarding the absorption of the drugs which were taken subsequently to it. It is well known that the pharmacodynamics of saline purgatives consists chiefly in the poor absorbability on the part of the intestinal walls of certain ions such as those of magnesium sulphate, phosphate, etc., and the accumulation of fluid in the intestines through the osmotic action

of the unabsorbed salt which, instead of being absorbed, actually draws more fluid into the intestinal lumen. This peculiar property of the saline purgatives considered in connection with the above described clinical experience led the authors to inquire into the effect, if any, that such saline purgatives might have upon the absorption and excretion of other drugs given by mouth simultaneously with or subsequently to the taking of the laxative.

PHENOLSULPHONPHTHALEIN

Administration by Stomach Tube.—One of the most convenient drugs for beginning such a study was thought to be the dye, phenolsulphonphthalein. This drug, as is well known, is rapidly and completely excreted by the kidneys irrespective of the method by which it may be administered. The rate of excretion can be easily determined quantitatively by the colorimetric method; hence its widespread use as a kidney function test.

Accordingly, this drug was administered to a number of dogs with a given quantity of water through the stomach tube. At the end of two and one half hours the dogs were catheterized and the amount of phenolsulphonphthalein excreted by the kidneys in the two and one half hours was determined colorimetrically. A few days later the same animals were given exactly the same amount of phenolsulphonphthalein in exactly the same amount of water as in the previous experiments, but on

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this occasion the water contained 5 per cent of sodium sulphate in solution. The fluid together with the dye and sodium sulphate was administered through a stomach tube as before, and the total output of phenolsulphonphthalein was again determined at the end of two and one-half hours. It was found that the amount of dye in the urine this time was less than one-half the amount excreted in the previous or control experiments. Similar experiments with phenolsulphonphthalein with and without sodium sulphate were performed on rabbits, with similar results; that is, it was found that the sodium sulphate solution markedly retarded the absorption and consequent excretion of the dye.

The same procedure was repeated on other occasions on both dogs and rabbits, but, instead of sodium sulphate, solutions of magnesium sulphate were used. It was found that this saline cathartic acted very much like sodium sulphate, in so far as it markedly retarded the absorption and excretion of phenolsulphonphthalein.

Experiments on Intestinal Loops.—In order to analyze the above phenomena more in detail, the following experiment was performed. A cat was anesthetized with ether, a laparotomy was made and the small intestines were exposed. Two loops of the small intestine, each 25 cm. long, were tied off, leaving the vascular supply intact. Into one of the loops there was injected 10 c.c. of a solution of sodium sulphate (10 per cent) plus 1 c.c. of phenolsulphonphthalein. Into the other loop was injected 1 c.c. of phenolsulphonphthalein in 10 c.c. of plain water. The abdominal wound was closed and the animal put in a cage. At the end of one hour the cat was killed with ether, the abdomen was opened again and the two loops of the intestine were examined. It was evident at once that one of the loops was greatly distended, like a sausage, while the other was collapsed. The two loops were excised and the fluid contents of each were measured. In the control loop, into which 10 c.c. of water had been injected, there were found only 1 or 2 c.c. of intestinal contents, whereas in the other loop, the one with the saline solution, the volume of the fluid had increased to over 30 c.c. Each loop of the intestine was washed out with water, the washings were combined with the fluid contents already obtained, and the amount of dye present in the intestinal loops was determined. It was found that whereas in the control over 50 per cent of the phenolsulphonphthalein had been absorbed by the intestine at the end of one hour, in the second loop there was present over 90 per cent of the dye. It was thus evident that the sodium sulphate had retarded markedly the absorption of the phenolsulphonphthalein from the intestinal lumen. This difference in absorption between the two loops was further strikingly corroborated by immersing each loop separately in a weak alkali. On treatment with the alkali, the mucosa of the normal loop became intensely red in color, showing that the dye had

penetrated inside the cells. On the other hand, the second loop showed very little pink discoloration, thus indicating that very little of the dye had been absorbed. On extracting each intestinal segment with alcohol, the phenolsulphonphthalein in the tissues themselves was extracted, and a much greater amount of dye was obtained from the control loop. Several such experiments were performed with similar results.

Intramuscular Injections.—It was then thought possible, though by no means probable, that perhaps the administration of saline purgatives by the stomach might exert some effect on the excretion of phenolsulphonphthalein even if the dye were given by injection. Accordingly, to settle this point experiments were made first on some rabbits. The usual kidney function test was performed on two rabbits by injecting each of them with 1 c.c. of phenolsulphonphthalein intramuscularly. At the same time each animal received 50 c.c. of water through the stomach tube. The urine was collected at the end of the first hour and at the end of the second hour and the amount of dye excreted was determined by the usual procedure. A few days later each of the same animals was again injected with 1 c.c. of phenolsulphonphthalein intramuscularly, and simultaneously with the injection there was introduced through the stomach tube about 50 c.c. of water containing 5 per cent of magnesium sulphate. The output of the dye was again determined at the end of the first and second hours, and it was found that the excretion of phenolsulphonphthalein was markedly delayed in the experiments in which the salines had been administered by stomach. Precisely similar results were obtained with sodium sulphate.

Following these studies on rabbits, similar experiments were performed on three dogs. Phenolsulphonphthalein was first injected intramuscularly and the kidney function of the dogs was determined by measuring the dye output at the end of the first and second hours. A few days later the same dogs were given the same amount of dye by injection, but before the injection each dog received by stomach tube a quantity of sodium sulphate. It was found in the latter series of experiments that there was a distinct diminution in the phenolsulphonphthalein output. Following these experiments some days later another normal kidney function test was performed on the same animals, and two days later the same animals were injected with the dye and magnesium sulphate was administered by stomach tube. In this case also the phenolsulphonphthalein output was diminished or retarded by the administration of the salines. From the above experiments it was evident that the administration of saline purgatives by stomach, tended to retard the excretion of phenolsulphonphthalein, not only when given by mouth but also after injection of the drug.

Observations on Man.—Inasmuch as the phenolsulphonphthalein test is a perfectly harmless one, and inasmuch

as saline purgatives can be taken with impunity by almost every one, a series of tests were made to discover whether the above experimental findings hold good for man. Accordingly, a number of observations were made on the authors themselves, their colleagues and friends. The results obtained indicated that the administration of saline purgatives, definitely and in most cases markedly, affected the kidney output of phenolsulphonphthalein. More detailed results of these experiments appear elsewhere.¹ In this place it is sufficient to state that the conclusions drawn from a study of phenolsulphonphthalein are of immediate clinical importance, as may be illustrated by the following case described to the authors by a genito-urinary surgeon.

Protocol.—A middle-aged man came to a physician for treatment of the bladder. In connection with a routine physical examination, a phenolsulphonphthalein test for kidney function was made and to the doctor's surprise and consternation it was found that the phthalein output for the first two hours was very low, about 16%, thus pointing to a derangement of kidney function. The patient in question was in the habit of drinking every morning a dose of a well known bitter water. During the course of treatment he became tired of taking the above bitter saline purgative and changed about to a daily dose of compound licorice powder. It happened that the physician soon after made a second kidney function test and to his surprise he found that the kidney output had improved and was 38%.

The above history is not an unusual one. In the present paper, however, the research undertaken deals not so much with the influence of saline purgatives on the absorption and excretion of drugs administered by injection as with experiments concerning the effect of salines on the absorption and excretion of various drugs introduced into the stomach and intestines. A large number of drugs were studied in this connection and the results obtained are summarized briefly in the following pages.

ABSORPTION OF POTASSIUM CYANIDE

In order to put the above experimental data as obtained with phenolsulphonphthalein to a crucial test experiments were made with solutions of the powerful and rapidly acting poison *potassium cyanide*. In these experiments cats and dogs were kept under anesthesia, laparotomy was performed, and a loop of the small intestine was tied off without interference with the mesenteric circulation. A blood pressure tracing and a tracing of the respiratory movements of the chest were recorded on a kymograph. After preparation of the animals, a given dose of potassium cyanide solution in a definite amount of water (10 c.c. of 5% solution of KCN) was injected into the intestinal loop and the blood pressure and respiration curves were studied from the beginning of the injection until stoppage of the respiration and the heart of the animal. The time of death was then noted. In another series of animals the same procedure was carried out with the exception that the potassium cyanide was adminis-

tered in a solution of sodium sulphate (10 c.c. of a 5% solution KCN in 10% Na_2SO_4). Blood pressure and respiratory tracings were taken again and the time of death was noted. It was found that, when potassium cyanide was given together with the sodium sulphate, its absorption and the subsequent picture of cyanide poisoning were greatly retarded, thus indicating that sodium sulphate tended to delay the absorption of the potassium cyanide by the intestinal mucosa. The following tracing illustrates one of the experiments. In this experiment potassium cyanide was injected into a loop of intestine together with sodium sulphate. It will be noted that the heart did not cease beating until about half an hour (36 minutes) after the injection of the poison. In control experiments, the same dose of cyanide injected in plain water solution produced death in about 7 minutes (Fig. 1).

In another experiment, after the injection of a 4% solution of potassium cyanide in 20 c.c. of sodium sulphate 10%, the animal lived 70 minutes, whereas the control dog, receiving the same quantity of cyanide in aqueous solution, died in seven minutes.

ABSORPTION OF CHLORETONE.

Chloretone was selected as representative of the hypnotic group of drugs. This drug has been used extensively in this laboratory as an anesthetic for cats and dogs. The effect of saline purgatives on the absorption of chloretone is most strikingly illustrated by the following experiments.

Exp. A.—A cat weighing 3 kilos was anesthetized with ether, laparotomy was performed and a loop of the small intestine, 20 cm. long, was tied off. Into this was introduced 100 mgms. of chloretone, per kilo weight of cat, dissolved in 20 c.c. of water. The abdomen was closed and the cat put in a cage. The effects of the ether anesthesia soon passed away but the anesthetic properties of chloretone quickly began to manifest themselves, so that before the end of an hour from the beginning of the experiment the animal was completely anesthetized by it.

Exp. B.—A cat weighing 3 kilos was anesthetized with ether as above, a loop of the intestines, 25 cm. long, was tied off, and into this was introduced a chloretone solution, 100 mgms. per kilo weight of cat, dissolved in 20 c.c. of a 5% solution of sodium sulphate. The cat was put in a cage. Effects of the ether soon passed away, but the anesthetic action of the chloretone did not manifest itself, and at the end of an hour the cat was sitting up quite contentedly. It was killed afterwards with ether.

ABSORPTION AND EXCRETION OF SOME OPIATES

Several opium derivatives were studied in this connection.

Apomorphin.—It is well known that apomorphin produces vomiting when introduced by mouth or injected into an animal, through being absorbed into the circulation and carried to the vomiting center in the medulla. A number of dogs were first given small amounts of apomorphin hydrochloride (30 mgms.) dissolved in 50 c.c. of water. The onset of vomiting after the administration of the drug was noted. This varied for individual dogs,



FIG. 1.—Experiment, June 7, 1921. Dog, 10 kilos. Ether anesthesia. Injected into intestinal loop 25 cm. long, 10 cc. of KCN 5% in a 10% solution of Na_2SO_4 . Death occurred at 4.30 P. M. Beginning of injection was at 3.54 P. M.

but was pretty constant for any one animal. The same animals were given the same doses of apomorphin in the same volume of fluid on another occasion. In the second series of experiments, however, instead of water, a solution of sodium sulphate (5%) was used as a vehicle. It was found that in the latter series of experiments vomiting came on much later and some times not at all.

Morphine.—To study the effect of salines on the absorption of morphine the bimeconate salt was found to be very suitable. A cat was anesthetized with ether and two loops of the small intestine of about the same length were tied off. Into one of the loops were injected 10 c.c. of water in which was dissolved 50 mgms. of morphine bimeconate. Into the other loop the same amount of morphine bimeconate was injected but dissolved in 10 c.c. of a 5% solution of sodium sulphate. At the end of one hour the cat was killed, each loop of intestine was carefully cut out, and the intestinal contents of each were measured. The control loop at the end of one hour contained less than 1 c.c. of fluid. The loop containing the saline purgative at the end of one hour was found to contain about 35 c.c. of fluid. The contents of each loop plus the washings of the mucosa were then examined for the content of morphine bimeconate. After first coagulating the proteids and filtering, a colorimetric determination of meconic acid with ferric chloride was made. It was found by this method that the specimen from the loop containing sodium sulphate gave a much deeper color reaction for meconic acid than the control loop, thus indicating that much less of the bimeconate had been absorbed in the first case than in the control.

Pantopon.—The effects of pantopon on the respiration were studied. A rabbit was trained to lie quietly on a board without anesthesia and its rate and depth of respiration were registered by means of a stethograph. This rabbit was then given through a stomach tube 20 mgms. of pantopon in 20 c.c. of water. The results obtained are illustrated by the following protocol.

Rabbit—2000 grams.

11:30 A.M.—Respirations—58 per minute	
11:45 A.M.—“ —56 “ “	
11:50 A.M.—“ —56 “ “	
11:55 A.M.—“ —56 “ “	
12 M. —“ —56 “ “	

Pantopon, 20 mgms. in 20 c.c. of water is introduced by stomach tube.

12:04 P.M.—Respirations—52 per minute	
12:08 P.M.—“ —50 “ “	
12:10 P.M.—“ —48 “ “	
12:16 P.M.—“ —46 “ “	
12:28 P.M.—“ —44 “ “ and very shallow	
12:35 P.M.—“ —40 “ “ “ “ “	
12:45 P.M.—“ —40 “ “ “ “ “	
12:55 P.M.—“ —40 “ “ “ “ “	

Exp. 2.—The same animal was used the following week and in this case the same amount of pantopon was administered by stomach tube, but mixed with 20 c.c. of a 5% solution of sodium sulphate.

Rabbit—2000 grams.

11:15—Respirations—52 per minute	
11:30—“ —50 “ “	
11:45—“ —50 “ “	
11:48—“ —introduced 20 mgms. of pantopon in 20 c.c. of sodium sulphate solution, 5%.	

12 M.—Respirations—50 per minute

12:15—“ —50 “ “	
12:30—“ —50 “ “	
12:40—“ —54 “ “	
12:45—“ —54 “ “	
12:55—“ —50 “ “	
1 —“ —50 “ “	
1:30—“ —50 “ “	
2 —“ —50 “ “	
2:30—“ —46 “ “ first effect of narcotic is noticeable.	
3 —“ —44 “ “	

The above protocols illustrate strikingly the difference in the narcotic action on the respiration due to the difference between the absorption of aqueous and saline solutions of pantopon.

OTHER ALKALOIDS

In addition to the opium derivatives a number of other alkaloids were tested. Among these were cocaine, strychnin, atropin and quinin.

Cocain.—The effect of saline laxatives on the absorption of cocain is illustrated by the following experiments.

Exp. Nov. 10, 1921.—Dog. 7.15 kilos. Ether anesthesia. Laparotomy was performed and a loop of the small intestine, 30 cm. long, was tied off. Into this loop were injected 10 c.c. of a 2% solution of cocain hydrochloride plus 20 c.c. of water. A blood pressure and respiration tracing was made and the first appearance of convulsions was noted. It was found that the cocain in this case very rapidly produced toxic symptoms, as indicated by failure in respiration, fall in blood pressure, and onset of tremors. Death occurred in 35 minutes after the injection of the alkaloid into the intestine.

Exp. Nov. 15, 1921.—Dog. 6 kilos. Ether anesthesia; laparotomy. A loop of small intestine, 50 cm. long, was tied off and into this were injected 20 c.c. of cocain-hydrochloride solution (2%) and 15 c.c. of a 5% solution of sodium sulphate. Blood pressure and respiration tracings were made and tremors and convulsions were looked for. There was no appreciable effect on the blood pressure or respiration or on the nervous system noted in the course of one hour from the time of injection. The dog was sacrificed later for another purpose.

From the above experiments it is evident that while in the control animal the loop was shorter in length and consequently the absorbing surface smaller and the amount of cocain injected was much less than in the saline experiment, nevertheless, toxic symptoms developed much sooner, thus very strikingly showing the inhibiting effect of sodium sulphate on the absorption of cocain hydrochloride.

Strychnin.—Experiments with strychnin were made on rabbits and dogs. The procedure was to tie off loops of intestine, inject a given dose of strychnin and note the first appearance of convulsions. It is hardly necessary to state that the experiments were performed under gener-

al anesthesia. It was found that when strychnin was administered in a solution of sodium sulphate the convulsions came on a little later than normally. Thus for instance, in one rabbit 4 mgms. of strychnin nitrate were injected into a loop of the small intestine, 15 cm. long, under paraldehyde anesthesia. The injection was followed by convulsions in 28 minutes. In another rabbit of the same weight the same dose of strychnin in a 5% solution of sodium sulphate was injected, and the convulsions did not set in until 35 minutes after injection. In dogs a similar difference in the time of onset of convulsions was noted. In all these experiments pains were taken to have the same degree of anesthesia in both the control and experimental animals, in order to eliminate differences in nervous response due to that source.

Atropin.—The effect of salines on the absorption of belladonna alkaloids is well illustrated by the following experiments.

Exp. May 11, 1922.—A cat was anesthetized with isopropyl alcohol by the method described by one of the authors elsewhere.¹ A loop of intestine, 20 cm. long, was tied off. Into this was injected a mixture of 4 c.c. of tincture of belladonna root plus 10 c.c. of water. The response of the heart to vagus stimulation with an induction coil was noted and the size of the pupils was also observed. It was found that the injection of belladonna produced a definite mydriasis in less than 5 minutes and paralyzed the vagus control of the heart within 10 minutes, after injection.

Exp. May 11, 1922.—A cat was anesthetized with isopropyl alcohol, as above. A loop of intestine, 25 cm. long, was tied off. Into this loop was injected the same amount of tincture of belladonna as above plus 10 c.c. of a 5% solution of sodium sulphate. The first appearance of mydriasis in this cat was noted after 15 minutes. The failure of vagus inhibition of the heart did not occur until after 20 minutes.

Quinidin.—Inasmuch as quinidin is employed extensively at present in the treatment of certain cardiac conditions, some experiments were made with this alkaloid. Equivalent amounts of quinidin sulphate were injected into loops of intestine, dissolved in water in some cases and in sodium sulphate solution in others. It was found by chemical tests that the absorption of this alkaloid was delayed by sodium sulphate. Very accurate quantitative determinations in this case, however, could not be made owing to the fact, emphasized by Lipkin² and others, that quinin and quinidin are rapidly decomposed in the intestinal canal. Ample evidence was nevertheless obtained to show a difference in absorption between the aqueous and saline solutions of the drug.

HEART DRUGS

Digitalis.—Some 15 experiments were made with digitalis on cats. A sample of digitalis tincture was first assayed by the cat method and its minimal lethal dose was found to be 0.86 c.c. per kilo. This tincture was used in some experiments as such, while in others it was evaporated to drive off the alcohol and diluted with saline to the original volume. Experiments were made first by administering a large dose of digitalis through

the stomach tube and determining the time of death with and without the addition of sodium sulphate. This method, however, was not found to be the most suitable on account of vomiting and other disturbances, and so resort was made to loop experiments. In these experiments long loops of the intestine were tied off and a dose of digitalis sufficient to kill the animal was injected. Observations were then made on the rapidity of exitus with and without saline purgatives. The experiments were made under ether or isopropanol anesthesia.² The following protocols will illustrate the findings.

Exp. A.—Cat, 2.2 kilos. Ether was given and then isopropyl alcohol. After laparotomy a loop of intestine, 55 cm. long, was tied off without interfering with the circulation. Into this 10 c.c. of the evaporated tincture of digitalis were injected together with 20 c.c. of water. The injection was made at 2:35 P. M. The cat was dead at 4:30 P. M. The volume of the contents of the loop after death was found to be 20 c.c.

Exp. B.—Cat weighing 1.8 kilos. Anesthetized as above. After laparotomy, a loop of intestine, 60 cm. long, was tied off. Into this was injected 10 c.c. of evaporated tincture of digitalis plus 10 c.c. of water plus 10 c.c. of a solution of sodium sulphate (3%). The time of injection was 2:50 P. M. The cat lived until 8:45 P. M. The contents of the loop after death was found to be 50 c.c.

Exp. C.—Cat, 2.0 kilos. Anesthetized as above. Loop of intestine, about 50 c.c. long, was tied off. Into this was injected 10 c.c. of evaporated tincture mixed with 15 c.c. of castor oil. Injection was made at 10:25 A. M. The cat died at 11:55 A. M.

The above experiments as well as others clearly brought out the fact that when digitalis was administered together with saline laxatives its absorption and consequent toxic symptoms took place much more slowly than when the drug was administered with water.

Convallaria.—Several experiments were made on cats and dogs with fluid extract of convallaria. This drug was found to be much more toxic than digitalis, but the relative absorption with and without sodium sulphate was the same as in the case of digitalis.

ANTIPIRETICS

Of this group of drugs quite a number were studied and numerous experiments were made both on animals and men because one of them had been the starting point of the whole research. The following antipyretics were examined; sodium salicylate, salol, aspirin, antipyrin, acetphenetidin and lactophenin.

Sodium Salicylate.—The test for this drug being very simple, experiments with it were made both on rabbits and man. The drug was administered to rabbits in some cases by stomach tube, in other cases by intraperitoneal injections, and the time of the first appearance of salicyl in the urine was noted. In man sodium salicylate was administered by mouth and the time of the first appearance in the urine was determined in the same way. The test for salicyl was made by the addition of ferric chloride and the characteristic violet color was obtained. In doubtful cases the urine was acidified and shaken with ether and the test with ferric chloride was

repeated. It was found, both in the case of rabbits and in the clinical cases, that the excretion of salicylate in urine was markedly delayed by the previous or even simultaneous administration of various saline purgatives, such as magnesium sulphate, sodium sulphate and sodium and potassium tartrate. It was not delayed however, by the administration of other laxatives of the non-saline variety, such as castor oil and cascara sagrada. The following experiments will illustrate some of the findings.

Exp. A.—Rabbit weighing 1800 grams was catheterized and the urine tested with ferric chloride with a negative result. At 11 A. M., 5 c.c. of a 2% solution of sodium salicylate plus 20 c.c. of water was introduced into the stomach through a sound. The urine gave a positive salicyl test at 11:45 A. M.

Exp. B.—Rabbit weighing 1800 grams, with negative urine test, was given at 11 A. M. 5 c.c. of sodium salicylate (2%) plus 20 c.c. of a 1.5% solution of sodium sulphate. The first positive test for salicyl in the urine did not appear until 2:40 P. M.

Exp. C.—Rabbit weighing 2000 grams. At 10:15 A. M. introduced into stomach 20 c.c. of a 3% solution of sodium sulphate. 10:45 A. M. introduced into stomach 2 c.c. of sodium salicylate (2%). 11:50 A. M. salicyl test—urine negative. 12:45 P. M. salicyl test—urine negative. 2:30 P. M. salicyl test—urine negative. 3:45 P. M.—urine negative. No positive test was obtained that afternoon.

Exp. D.—Rabbit weighing 1500 grams, injected with 2 c.c. of a 2% solution of sodium salicylate intraperitoneally, at 3:05 P. M. Positive test for salicyl in the urine was obtained at 4:15 P. M.

Exp. E.—Rabbit weighing 1800 grams injected with 2 c.c. of sodium salicylate (2%) intraperitoneally at 3:15 P. M. Urine gave positive test for salicyl at 4:20 P. M.

Exp. F.—Rabbit weighing 2000 grams given 5 c.c. of cascara sagrada tincture in water by stomach tube, and this was followed by an intraperitoneal injection of 2 c.c. of a 2% solution of sodium salicylate, at 2:45 P. M. Urine gave a positive test for salicyl at 4:10 P. M.

Exp. G.—Rabbit weighing 1800 grams was given 10 c.c. of castor oil by stomach tube. 15 minutes later injected with 2 c.c. of a 2% solution of sodium salicylate intraperitoneally. Urine gave a positive test in one and a half hours.

Exp. H.—Rabbit weighing 1500 grams. At 1 P. M. given 15 c.c. of a 5% solution of sodium sulphate by stomach tube and simultaneously given an injection of 2 c.c. of sodium salicylate (2%) intraperitoneally. First indication of salicyl in the urine did not appear until 4 P. M.

Salol.—Experiments with phenyl salicylate, or salol, were made on man. As is well known, when this drug is administered by mouth, a positive test for salicyl is normally given in about an hour, to an hour and a half. It was found that the absorption and consequent excretion of salol was delayed by the administration of saline laxatives. Thus one of the authors was found to excrete the drug normally in a little over an hour. The same subject when taking salol after a previous dose of magnesium sulphate ($\frac{1}{2}$ ounce) did not give a positive urine test for nearly three hours.

Aspirin.—Experiments with aspirin or acetylsalicylic acid were made on animals and on man. It was found that the absorption of the drug was markedly delayed by the previous and even simultaneous administration of

saline purgatives. In order to make sure of the first appearance of the salicyl after this drug, the tests for it were made with special care. In all cases whether dealing with urine or with other fluids the specimens were first hydrolyzed by the addition of a little sodium hydroxide and boiling, in order to make sure of breaking up all the aspirin into salicylic acid. Solutions were then carefully titrated and made very slightly acid and then definite quantities of ferric chloride were added.

The effects of salines on the absorption of aspirin are most strikingly illustrated by loop experiments. Cats were used in these experiments. Under anesthesia the abdomen was opened and two loops of the small intestine of approximately the same length were tied off. Into one of these 0.3 gram of aspirin suspended in water was introduced through a funnel and the loop was tied off. Into the other loop the same quantity of aspirin was introduced with the same amount of fluid but for this second loop instead of water a solution of sodium sulphate (2.5%) was used. The abdomen was closed and the cat was killed an hour later. The contents of each loop were carefully collected and measured. It was found in every case in which the aspirin was introduced in aqueous solution, that most of the fluid at the end of one hour had been absorbed. In the loops into which aspirin was introduced with sodium sulphate, the volume of fluid at the end of one hour instead of being decreased was actually increased to two or three times the original volume. The contents of each loop plus the washings of each loop were examined chemically for the amount of aspirin remaining in each and the quantity of the drug in each loop was compared colorimetrically. It was found that at the end of one hour about three times as much aspirin was absorbed from the loop with water than from the loop into which the aspirin was introduced with sodium sulphate. In other experiments, similar differences in absorption were found even after two hours.

Experiments with aspirin on man yielded very similar results. These experiments were made on a large number of students and also on the authors themselves. Aspirin was administered alone with some water, and some days later the same subjects took the same amounts of the drug with saline purgatives, such as magnesium sulphate, sodium sulphate, a Seidlitz Powder, or sodium phosphate. It was found that in most cases normally the first appearance of the salicyl in the urine occurred within one hour after administration of the drug. When, however, the aspirin was taken after a saline purgative, no positive test could be obtained from the urine within anywhere from three to six hours or longer. In fact, when the experiment was performed in the afternoon, no positive test could be obtained on that day. No such effect was noted after purgatives of the non-saline type.

Antipyrin.—A few experiments were made with antipyrin on man and on other animals. The drug was

detected by means of the ferric chloride reaction, which gives a red color, and the isonitroso test made by the addition of sodium nitrite, acidifying with sulphuric acid and shaking out with chloroform. Experiments on intestinal loops were performed with and without sodium sulphate and clearly indicated that the absorption of the drug was markedly delayed by the administration of sodium sulphate. The same phenomenon was noted after the administration of antipyrin by mouth to man.

Lactophenin.—The following experiment with lactophenin will serve to illustrate the effect of saline laxatives on the absorption of antipyretics belonging to the phenetidin group. Two loops of intestine were made in a cat and into each of these 50 mgms. of lactophenin was introduced, into one together with water, into the other together with a solution of sodium sulphate. After one hour the contents of each loop were tested for indophenol, by boiling with concentrated hydrochloric acid and treating with chromic acid. It was found that the sodium sulphate markedly delayed the absorption of the drug.

POTASSIUM IODIDE AND HEXAMETHYLENAMINE

These two drugs, it is well known, are among those which are most rapidly excreted by the animal organism. Not only are they very rapidly absorbed but they also very soon appear in most of the secretions of the body. In the urine hexamethylenamine can be detected within 20 minutes, while iodides appear in the urine in less than 10 minutes after ingestion. It was therefore with special interest that experiments were made with both of these drugs on man.

Hexamethylenamine (urotropin) was administered to a large number of normal subjects in doses of 0.5 gram by mouth and the time of the first appearance was ascertained. The same subjects on another day were given the same drug together with or following a dose of saline laxative and the time of the first appearance in the urine was compared with the normal. The test used was the so-called Hehner's test which detects both hexamethylenamine and free formaldehyde. It is performed by taking 0.5 c.c. of the urine and mixing it with an equal quantity of fresh milk and then stratifying it with 1 c.c. of 50% sulphuric acid containing a trace of ferric chloride. A violet ring very soon appears. It was found that, whereas normally after the ingestion of 0.5 gram of urotropin its excretion could be detected in the urine in about 15 minutes, the same subjects when taking the drug with a saline purgative showed a delay in the excretion. This delay was in some cases not very remarkable but in other subjects it was very striking, as may be illustrated by the following protocols.

Exp. D. M.—Given 0.5 gram urotropin together with a Seidlitz Powder. The first appearance of a positive test in the urine was 2½ hours after the administration of the drug. The normal time of appearance of the drug in the same subject was 20 minutes.

Exp. N. W.—Normal excretion of urotropin was 15 to 20 min-

utes. The subject was given half an ounce of magnesium sulphate and this was followed by 0.5 gram of urotropin. A faintly positive test was obtained 2 hours after administration.

Exp. C. K.—Normal excretion of urotropin about 20 minutes. Excretion after half an ounce of magnesium sulphate was 1½ hours later.

The experiences with *potassium iodide* were not so striking as those with urotropin. Twenty minims of the saturated solution were given to various subjects with and without saline purgatives. The normal excretion of iodide could be detected within 15 minutes. After the previous administration of magnesium sulphate a slight delay was observed. Thus, in one subject the first positive indication of iodide in the urine was one hour after ingestion. In another it was 45 minutes. In still others the drug could be detected in half an hour after taking by mouth. The test employed was by the addition of 0.5 c.c. of a 2% solution of sodium nitrite, acidifying with 0.5 c.c. of dilute sulphuric acid and then shaking out with chloroform. A positive test was indicated by a pink or violet coloration of the chloroform.

ANTISEPTICS

Phenol.—Several years ago one of the authors in studying lavage in acute carbolic acid poisoning showed that the most suitable solution for lavage in acute carbolic acid poisoning was a saturated solution of sodium sulphate.⁴ In the light of the present investigation the results obtained in this toxicological study of carbolic acid poisoning were not surprising. The sodium sulphate undoubtedly served two purposes: it not only served as a solvent for the washing out of the carbolic acid, but it also acted as a purgative and, above all, as an agent which tended to *prevent* the further *absorption* of the poison. This experiment with sodium sulphate solution in the treatment of carbolic acid poisoning prompted the authors in the present investigation to make some observations on the effect of saline purgatives on the absorption of mercuric chloride.

Bichloride of Mercury.—Two sets of experiments were made. In one series rabbits were used. To some of these an aqueous solution of mercuric chloride was administered by stomach tube. To others the same quantity of bichloride, proportionate to weight, was administered together with or following a previous administration of sodium sulphate. It was found, when a concentrated solution of saline was employed, that the animals succumbed more quickly in the first series of experiments than in the second. In other words, the sodium sulphate seemed to retard the absorption of the mercury and promote its elimination by purgation. The following two protocols may serve as illustrations.

Exp. Dec. 21, 1921.—Rabbit A, weighing about 2000 grams, was given by stomach tube 125 mgms. of mercuric chloride in 20 c.c. of water. The animal died in 12 hours.

Exp. Dec. 21, 1921.—Rabbit B, weighing about 200 grams, was given by stomach tube 125 mgms. of mercuric chloride in 20 c.c. of 7.5% sodium sulphate solution. The animal was alive on the following day, Dec. 22nd, when a similar dose of bichloride in sodium sulphate was administered. The rabbit died a day later.

In some of the experiments on rabbits the bladder was catheterized and an attempt was made to detect the presence of mercury in the urine. The method employed was similar to those described by Vogel and Lee⁵ and later by Elliott.⁶ A spiral of copper wire was placed in the urine and concentrated hydrochloric acid, 10 c.c. in 200 c.c. of urine, was added. The flask was thoroughly shaken and allowed to stand over night. The copper wire spiral was then heated in a small hard glass tube and any mercury that might be adherent to it was volatilized and allowed to deposit on gold foil. By this method the experiments seemed to indicate that more mercury was absorbed and consequently excreted in the urine of the control animals than in the case of those which had had a previous dose of sodium sulphate. Inasmuch as these experiments, however, were not very satisfactory, a quantitative study of the absorption and excretion of bichloride was made on cats by means of intestinal loops. Three such experiments were performed and in each case the figures obtained clearly indicated that the sodium sulphate distinctly retarded the absorption of the bichloride. The following protocols illustrate well these findings.

Exp. Dec. 26, 1921.—A.—A cat weighing about 3 kilos was anesthetized with ether. The small intestine was tied off just below the pylorus and again above the ileocecal valve. Into this intestine was injected 50 c.c. of water containing 450 mgms. of mercuric chloride. The abdomen was closed and the animal was kept narcotized until its death which occurred in about three hours. The intestine was then cut out, its contents collected and the surface of the intestine thoroughly washed. The washings were mixed with the intestinal contents. The total volume of fluid was examined quantitatively for mercury.

Exp. Dec. 26, 1921.—B.—Another cat weighing about 3 kilos was treated in the same way as above. The whole small intestine was tied off above and below and 450 mgms. of bichloride were injected in 50 c.c. of 5% solution of sodium sulphate. This experiment was begun about 11 A. M. and the animal was still living narcotized quite late in the afternoon. The exact time of death was not noted, but the cat was found dead the following morning. The whole small intestine in this case was also cut out and the contents and washings preserved and worked up for mercury.

The macroscopical appearance of the intestines in Experiments A and B were quite different. The mucosa in cat A showed violent irritation. It was extremely congested and hemorrhagic and contained a bloody mucus. In cat B the intestinal mucosa appeared more normal. It was much paler than in the previous experiment and did not show so much irritation or hemorrhagic exudate.

Chemical tests for mercury were made by first treating the intestinal contents of each cat with concentrated hydrochloric acid and a little potassium chlorate. The solutions were boiled for several hours until all traces of

chlorine had been driven off. They were then diluted in each case to the same volume. The solutions were then divided into two parts and examined for mercury by two independent workers. The mercury was precipitated with hydrogen sulphide and filtered through Gooch filters. The sulphide was then weighed. The quantity of mercuric sulphide obtained in Experiment A was 105.6 mgms. an amount equivalent to 123.24 mgms. of mercuric chloride. The mercuric sulphide obtained from the intestinal contents of cat B was 141.2 mgms., an amount equivalent to 164.78 mgms. of mercuric chloride. The difference between the above figures indicates that less mercury was absorbed from the intestine containing the sodium sulphate than from the one in which the bichloride had been introduced in aqueous solution. Two other sets of experiments on cats yielded similar quantitative differences.

DISCUSSION

The experiments described above are quite sufficient to establish beyond doubt that saline purgatives can and do affect markedly the absorption and consequent excretion of other drugs. Bearing in mind the pharmacodynamics of saline purgatives such a result is not altogether surprising when both the saline and other drugs are administered by mouth or introduced into the intestinal lumen. More remarkable, however, are the findings in the case of *injections* such as were made with phenolsulphonphthalein in man and in other animals and with sodium salicylate in rabbits. It is evident that the absorption and excretion of drugs are influenced to some extent by saline laxatives even when such drugs are given by injection after the purgatives have been previously administered by stomach tube. The explanation of this phenomenon is not altogether clear. Undoubtedly, however, it must have something to do with the concentration of the blood and the distribution of fluids in the body. It has been known for some time that saline purgatives will concentrate the blood, as is indicated by the blood count, and this has been recently emphasized by the work of Underhill.⁷ This probably plays a rôle in the phenomena studied in this paper. The findings obtained are of an immediate practical interest. It is evident that it is not immaterial whether drugs are administered with or without certain cathartics. The salines will markedly retard the absorption of many drugs, such as antipyretics, digitalis, etc., and the therapeutic results expected in clinical cases may consequently not be attained. The authors have studied the relation of other laxatives such as calomel, cascara sagrada, castor oil, etc., in this connection but have not found any important influence exerted by these drugs on the absorption and excretion of other medicaments. The exact *duration* of the inhibitory effect of saline purgatives on the intestinal mucosa has not been made a subject of exhaustive study by the authors. It will, of course, vary with the drugs and the

purgatives used and also with the individual subject. This inhibitory or retarding influence on the absorption of medicaments should, however, be born in mind by the careful clinician who may wish to obtain a prompt response on the part of the patient to the effects of any given medication.

Another important bearing which the above findings serve to reveal is the usefulness of saline purgatives and lavage with saline purgatives in cases of poisoning. Such a procedure is certainly an innocuous one and in the light of the present investigation would tend to minimize the absorption and promote the expulsion of any toxic substances remaining in the intestines.

SUMMARY

1. The absorption of a large number of drugs from the gastro-intestinal tract was studied by chemical and physiological methods in animals and in man.

2. It was found that all the drugs examined were markedly delayed in their absorption from the stomach and intestines when they were administered subsequently to or even simultaneously with saline laxatives.

3. The absorption and subsequent secretion of phenol-sulphonphthalein and sodium salicylate were markedly

affected by saline purgatives, even when the former drugs were administered by intramuscular or intraperitoneal injection.

4. Laxatives other than those of the "saline" type exerted no important effect on the absorption of the drugs from the stomach and intestines.

5. The above findings have an important practical bearing on the therapeutic administration of medicaments.

6. The above experiments, especially those with phenol and mercuric chloride, furthermore, speak in favor of a more extensive employment of saline laxatives in the treatment of toxicological cases.

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LOCALIZATION OF CUTANEOUS NERVES BY ELECTRICAL STIMULATION, APPLIED TO NERVE-BLOCK ANAESTHESIA

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In a report published in the Anatomical Record,¹ the author described a new method of teaching the distribution of cutaneous nerves to students in Gross Anatomy. This procedure, suggested by the work of Trotter and Davies² on cutaneous sensibility, consists of stimulation of the cutaneous nerves through the skin with a unipolar electrode. By this means, the point of emergence of the cutaneous nerve from the deep fascia and the subsequent course can be easily identified; the area of distribution of the nerve is clearly outlined by the tingling sensation.

With the rapidly increasing use of local anaesthesia in surgical procedures, of all kinds, methods have become pretty well standardized. Any suggestions, therefore, can now be expected only to add some refinement to a technic which in expert hands is already nearing perfection. The intelligent use of local anaesthesia in its wide field of application requires a highly specialized knowledge of the distribution of all cutaneous nerves. Though any region may be affected by the use of paravertebral nerve-block, this method is not always justified by the scope of the surgical procedure. Simple regional anaesthesia has many advantages over the more generally used infiltration-method which need no exposition here, and has been widely used since the publication of Cush-

ing's paper in 1900.³ It is to the former type of anaesthesia that this method of accurate localization of nerve trunks can be applied.

The suggested method in no way absolves the operator from the necessity of being familiar with the general position of the nerve trunks. No amount of knowledge, however, can prepare one for the individual variations which are constantly met with in the position, point of emergence from the fascia, and above all the ultimate distribution of superficial cutaneous nerves. These variations may occur even on opposite sides of the same individual and undoubtedly are responsible for many of the unsatisfactory results obtained in block anaesthesia.

To meet these difficulties such a localization of cutaneous nerve trunks furnishes a method of almost absolute accuracy. A single dry cell, a small induction coil,* and an indifferent and a stimulating electrode constitute the entire apparatus needed. A faradic current too weak to cause muscular contraction will give the tingling sensation over the entire distribution of the nerve when the stigmatic electrode is applied immediately over the nerve trunk. When the anatomical position of the nerve trunk

* Harvard Instrument Company.

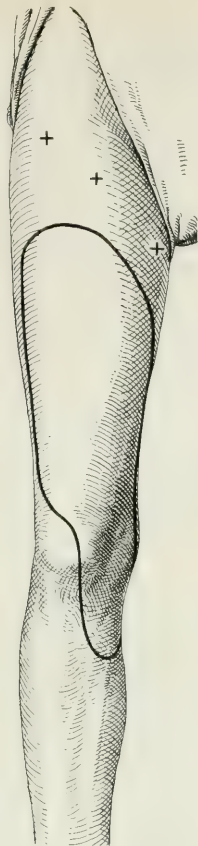


FIG. 1.—Localization of Cutaneous Nerves by Electrical Stimulation, Applied to Nerve Block Anæsthesia.

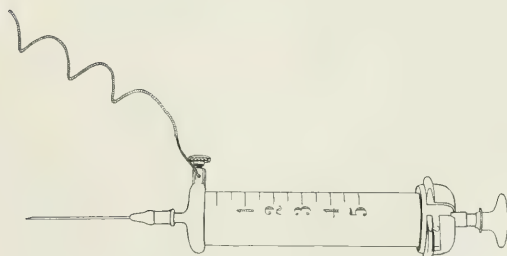


FIG. 2.—Localization of Cutaneous Nerves by Electrical Stimulation Applied to Nerve Block Anæsthesia.

is abnormal or when there is some uncertainty as to its exact location, careful search with the stigmatic electrode over the expected area invariably reveals the nerve. Sensory nerves over practically the entire body can be located in this way.

Having determined the size of the area to be anesthetized and located the nerve trunks supplying the region, the injection of novocain, or any other appropriate drug, at the points determined will give the required anaesthesia over the entire area of the cutaneous nerve.

With this accurate localization the point may be marked on the skin † at any time previous to the operation, as in the ward by the interne, and the surgeon is required simply to inject the anæsthetic at the points indicated. Smaller amounts of the anæsthetic are sufficient when injection is thus made either directly into the nerve sheath or into its immediate vicinity. The illustration (Fig. 1) is made from a case in which 0.5 c.c. of novocain was injected at each of the points marked by a star. The resultant cutaneous anaesthesia involved the whole anterior surface of the thigh, as shown by the black outline, and lasted for a period somewhat over two hours.

Even greater accuracy may be obtained when injecting the anæsthetic by using the hypodermic needle as the stimulating electrode, a suggestion made by Dr. H. H. Young. This can readily be done by simply soldering a fine copper wire to the needle or by the use of a specially prepared hypodermic syringe as shown in Figure 2. To the outlet of the syringe there has been attached a small set screw which readily permits making the necessary connection with the induction coil, and in addition does away with the somewhat awkward needle and wire; the latter however has the advantage of being very easily made. It is not necessary to insulate the needle when used in this way. After insertion of the needle through the skin at the pre-determined points, a weak current is passed through the needle as stigmatic electrode and the nerve trunk located with remarkable accuracy. Deviation of the needle-point less than a millimetre to either side of the nerve trunk will cause a loss of the stimulating

effect. When the nerve has thus been positively identified, the anæsthetic may be injected. Prompt dulling and then complete loss of sensation results. The procedure is in no way painful. Actual stimulation of the nerve trunk with this mild current causes no more intense sensation than stimulation through the skin. This method is also applicable to nerve trunks lying deep in the muscle tissue before they have emerged from the fascia.

Of course, no new principle is involved in the method suggested; it is simply a refinement of one branch of the general technic of producing local anaesthesia by the injection of a drug in the region of the nerve trunk. This method has not been used for actual clinical purposes but sufficient direct observations have been made to indicate that it can have definite practical use in a great variety of surgical procedures. This is particularly true as regards the extremities and the neck. A certain amount of intelligent cooperation on the patients' part is necessary but this can readily be obtained by first demonstrating the sensation to be expected by stimulating some obvious trunk such as the ulnar or radial.

CONCLUSIONS

Localization of sensory nerve trunks by unipolar stimulation through the skin furnishes a method whereby nerve-block anaesthesia can be produced with the greatest accuracy and certainty. Difficulties due to anatomical variations are done away with and the surgeon is enabled to deal with each case individually. The apparatus required is easily prepared and the procedure is in no way painful or unpleasant to the patient.

FIGURE LEGENDS

FIG. 1.—Crosses indicate position of nerve trunks and points of injections of anæsthetic. Black line outlines area of anaesthesia obtained.

FIG. 2.—Record syringe fitted with set screw for attachment of wire.

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† We have used an excellent indelible skin-ink for this purpose, described by N. S. Finzi, *Brit. M. J. I*, 52, Jan. 12, 1918.

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THE EFFECT OF SODIUM GERMANATE UPON THE TOTAL HEMOGLOBIN OF THE ALBINO RAT

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In a previous publication it has been shown that germanium dioxide in alkaline solution is non-toxic and non-corrosive for the tissues of the albino rat when administered by subcutaneous injection.¹ It has also been pointed out that such a solution will cause a marked increase in the number of erythrocytes in the peripheral blood when administered in the same manner.² A further study has demonstrated that this rise in the red blood corpuscles is accompanied by an increase in the number of nucleated red cells in the bone-marrow of these animals, indicating that stimulation of the erythrocytogenic tissue has taken place as the result of the action of this compound.³

The physiological action of germanium compounds is, at present, almost wholly unknown. In spite of the evidences of increased bone-marrow activity obtained from the examination of histological preparations, it was thought possible to settle the question of a real or an apparent increase in the number of the erythrocytes in the blood. If there were a real increase in the number of erythrocytes, the total hemoglobin should be higher in treated animals than in normals, providing the hemoglobin parallels the red cell count.

The purpose of the present study was to determine what change, if any, takes place in the total amount of hemoglobin in the blood of the albino rat after the administration of germanium dioxide in alkaline solution.

EXPERIMENTAL

One gram of chemically pure germanium dioxide was sifted into 200 milliliters of distilled water. Sufficient normal sodium hydroxide was then added to dissolve the oxide, and make the solution distinctly alkaline. The excess of alkali was neutralized by the addition of enough normal hydrochloric acid to bring the solution back to a pH of 7.0-7.2. The solution was then sterilized by boiling, and after cooling the volume was made up to 250 milliliters by adding sterile distilled water. The addition of the alkali converted the dioxide into sodium germanate according to the equation:



As will be shown in a future paper, it is in this form that germanium is most efficient as an erythropoietic agent. The addition of an excess of alkali is therefore imperative.

Two series of mature rats were selected, one series of seven animals being used as tests, and the other series of six reserved as controls. Red blood cell counts and hemo-

globin estimations were made on the rats of both series, and immediately after these were completed, the test rats were injected with a dose of 17 milligrams of the sodium germanate solution per kilo of body weight. This is equivalent to a dose of 10 milligrams of the dioxide per kilo.

For a period of three weeks preceding, and during the course of the experiment, the rats received the same varied diet, in order to safeguard against any variations in the action of the germanate solution that could arise from dietary influences. The work of Hooper, Kolls, and Wright⁴ shows that fasting and various diets will strongly influence the action of arsenic compounds; and it is quite possible that the same factors have an influence on the action of other substances.

One week after the initial counts and hemoglobin determinations were made, the red cell counts were repeated and the hemoglobin redetermined on the rats of both series. Immediately after this was done, total hemoglobin determinations were made on all the animals, employing the following technique which is a modification of the method described by Jaquet and Suter.⁵

The rat was placed under ether anaesthesia and tied to a small animal board by its four extremities. The board was fastened upright over a large beaker in such a manner that the blood and perfusing fluid would drip directly and without loss into the vessel. The ventral surface of the rat was then washed with a five per cent solution of sodium citrate in order to prevent the blood from clotting on the animal's fur. The abdominal and thoracic cavities were then opened by a large inverted T-shaped incision. These cavities were immediately flooded with the sodium citrate solution in order to lessen the tendency toward clot-formation.

While the heart was yet beating, the left ventricle was opened, and a cannula was inserted through the ventricle and into the aorta, and held firmly in place by a ligature. Precaution was taken not to allow the tip of the cannula to pass into the subclavian or carotid arteries, as this would cause an incomplete perfusion. A clamp held the cannula in position and kept its weight from dragging the aorta out of place. The hepatic vein was then cut at its site of emergence from the liver, and the perfusing fluid, physiological saline, was allowed to pass through the animal's vascular system. As soon as the fluid had begun to circulate, the rat was suspended by means of a cord tied around its upper incisors, and the ligatures were removed from its extremities, in order to facilitate the return flow of blood from the feet. The reservoir con-

taining the perfusing fluid was placed at a height of two meters above the animal's heart.

The perfusion was allowed to continue until the fluid leaving the animal was colorless. By this time the liver and kidneys were very pale in color and practically bloodless. An interval of from two and one-half to three and one-half hours was necessary to accomplish this, and from 1000 to 1400 milliliters of the perfusing fluid were required to complete each perfusion.

The blood volume of the rat was then estimated by means of Hatai's formulæ⁶ which are based on the work of Chisolm.⁷ These formulæ are as follows:

1. For rats under 150 grams body weight, (Both sexes)
 $\text{Blood volume, (in c.c.)} = (\text{Body weight in grams})^{0.9} \times 0.099.$
2. For male rats 150 grams and over,
 $\text{Blood volume, (in c.c.)} = (\text{Body weight in grams})^{0.9} \times 0.09608.$
3. For female rats 150 grams and over,
 $\text{Blood volume, (in c.c.)} = (\text{Body weight in grams})^{0.9} \times 0.10494.$

On the basis of this estimation sufficient distilled water and hydrochloric acid were added to the perfusate to obtain a final dilution of one part of blood in 250 parts of tenth-normal hydrochloric acid. The action of the hydrochloric acid converted the hemoglobin into acid hematin. The estimation of the total hemoglobin was made directly from this dilution, using the permanent glass standard described by Newcomer.⁸ The preliminary hemoglobin determinations were made by the same method.

In this experiment the red cell counts were determined from samples of blood drawn from the tip of the animal's tail. This method is, in the writer's opinion, the method of choice for the purposes of the present study, and preferable to the method of obtaining the sample of blood directly from the heart of the rat. The danger of puncturing the pleura is very great in such a small animal, and as Bürker, Ederle, and Kircher have shown,⁹ the production of a unilateral artificial pneumothorax causes a true polycythæmia, and such an accident would nullify the results of his experiment.

RESULTS

The results are summarized in the tables.

Following the injection of the germanate solution there was a marked rise in the number of erythrocytes in the peripheral blood of the test rats.

It will be seen from the tables that in the control animals, the hemoglobin readings, as determined by using a measured quantity of blood, and those obtained from the perfusate diluted on the basis of Hatai's formulæ, are consistent. This would indicate that for the normal rat, the blood volume may be estimated within a reasonable degree of accuracy by the use of these formulæ. In the

test rats, however, on comparing the hemoglobin readings obtained by the two methods, considerable discrepancy was noticed, as the estimated concentration of hemoglobin obtained from the total hemoglobin determination was always higher than that obtained from a measured sample of blood. It was apparent, therefore, that Hatai's formulæ could not be used to estimate the blood volume of the rats treated with the germanate solution. The most plausible explanation of the discrepancies between the partial and the total hemoglobin determinations in these animals is the assumption that an increase in the blood volume has occurred.

This experiment, then, furnishes an additional evidence that the increase in erythrocytes following the injection of sodium germanate is due to a true erythropoiesis and is not a relative effect due to a simple concentration of the blood plasma, such as Bertelli, Falta, and Schweeger,¹⁰ and Lamson¹¹ have shown to occur after the administration of epinephrin. The results of the present study show clearly that no concentration of the blood plasma has occurred, for if such were the case, the concentration of hemoglobin based on a normal blood volume would be lower in the total hemoglobin determinations. Since these, on the contrary, show a distinct tendency to run higher than the corresponding readings obtained from a measured amount of blood, there is furnished a strong indication that the blood volume is increased following the injection of sodium germanate solution.

SUMMARY AND CONCLUSION

Two series of albino rats were taken. One lot of seven animals was used as tests, and the other series was reserved as controls. Red cell counts and hemoglobin estimations were made on all, and the test rats were then injected with 17 milligrams of sodium germanate per kilo of body weight. One week later red cell counts and hemoglobin readings were done again on all. The rats of both series were then perfused and total hemoglobin estimations were made from the diluted perfusates.

A study of the results shows that in the controls there was no significant change in the red cell counts, in the hemoglobin concentration, or in the blood volume. In the test animals there was a marked increase in the number of erythrocytes per cubic millimeter of blood, a moderate increase in the concentration of the hemoglobin, and a variable increase in the blood volume. There was no evidence of a concentration of the blood plasma in any of the animals.

It is therefore concluded from this and the preceding papers that the increase in the red cells of the blood of the rat following the injection of sodium germanate is in all probability due to a stimulation of the bone-marrow and not an effect which follows a concentration of the plasma.

TABLE I.—CONTROLS.

First Determination.					Second Determination.				
	Weight in grams	R. B. C. count, in millions	Hemoglobin, gms. per 100 c.c.		Weight in grams	R. B. C. count, in millions	Hemoglobin, gms. per 100 c.c.	Total Hemoglobin, gms. per 100 c.c.	Blood volume, c.c., calculated from Haas's Formula
1.	167.	9.00	10.63	177.	9.25	9.94	10.09	9.62	9.72
2.	188.	8.10	12.01	195.	8.28	11.73	11.83	11.06	11.16
3.	179.	7.97	11.73	181.	8.00	11.73	12.10	10.34	10.66
4.	241.	8.43	11.87	245.	8.56	11.73	11.59	13.58	13.42
5.	137.	8.52	11.04	137.	8.73	11.45	11.65	8.29	8.43
6.	232.	8.50	10.35	240.	8.64	9.73	10.74	13.32	13.82
Mean.	190.6	8.42	11.27	195.8	8.54	11.05	11.33	11.03	11.20

TABLE II.—TESTS.

First Determination.					Second Determination.				
	Weight in grams	R. B. C. count, in millions	Hemoglobin, gms. per 100 c.c.		Weight in grams	R. B. C. count, in millions	Hemoglobin, gms. per 100 c.c.	Total Hemoglobin, gms. per 100 c.c.	Blood volume, c.c., calculated from Haas's Formula
1.	256.	8.30	10.35	260.	9.93	11.04	16.32	14.29	21.13
2.	240.	7.84	10.76	253.	9.12	11.73	12.70	15.17	16.42
3.	219.	7.64	10.90	228.	9.18	12.01	12.77	13.90	14.96
4.	192.	8.06	11.73	190.	9.24	12.42	13.81	10.80	12.01
5.	200.	9.28	12.14	215.	10.07	12.42	13.62	11.79	12.92
6.	113.	7.27	10.63	124.	9.62	11.32	12.67	7.58	8.48
7.	148.	7.74	10.21	161.	9.58	11.04	13.44	9.30	11.32
Mean.	195.4	8.02	10.96	204.4	9.52	11.71	13.62	11.83	13.89

The two determinations were made one week apart.

The sodium germanate was injected into the test animals immediately after the first determination.

I wish to thank Dr. A. C. Kolls for many helpful suggestions.

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GIANT CENTROSFERES IN XANTHOMATOUS TUMORS

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When normal cells begin to degenerate in tissue-cultures a series of very characteristic but complicated changes takes place. The mitochondria break up into fine granules, form vesicles, or disappear entirely (Lewis and Lewis, 1915);¹ in some cells there is a great accumulation of granules and vacuoles which stain with neutral red (W. H. Lewis, 1919),² while in others the most striking feature is the formation of a giant centrosphere (W. H. Lewis, 1920).³ It is with the evidence of degeneration afforded by the latter that we are concerned in this communication. The centriole in tissue-culture cells that have been fixed and stained seems always to be in the form of a granule, single or double, usually the latter. The peculiar differentiated region around the centriole is called the centrosphere and corresponds to the central body, the aster, or astrosphere of the dividing egg and cell (W. H. Lewis, 1920).³ This structure is at one side or one end of the nucleus and generally in direct contact with it. Dr. Lewis found that the mitochondria and

degeneration granules have a marked tendency to arrange themselves around the centrosphere and to move in radiating paths to and from this structure. The pigment in true pigmented cells, as well as pigment granules that have been taken in as foreign bodies, shows the same peculiar arrangement about the centriole and centrosphere. When the cell begins to degenerate under certain conditions, the centrosphere increases enormously in size, becoming as large as or even larger than the nucleus. In this giant centrosphere Dr. Lewis distinguishes a centriole, single or double, immediately surrounded by a clear medullary zone, and beyond this a larger cortical zone. The granules and vacuoles are arranged about this outer cortical zone.

In studying a group of xanthomatous tumors which contained many giant cells, much blood pigment from other cells, and numerous foam cells, the writer noted that the granules in the pigment-bearing cells were arranged about a clear area, often the size of the nucleus

or a little larger, which occupied the center of the cell and displaced the nucleus to one side (Fig. 1). By careful focusing it could be determined that this clear area was a spherical body, one portion of which was in direct contact with the nucleus (Fig. 2), and that around this sphere was an outer shell of granules, complete except where it came against the nucleus. Often the cells were so filled with granules as to obscure all other cellular structures (Fig. 1). In the sections from which the figures here shown were taken (Surg. Path. No. 3890), nearly all the pigmented cells contained these clear central areas. A few cells showed an accumulation of granules to one side of the nucleus without the formation of this area (Fig. 3).

In several types of cells in tissue-cultures the neutral red granules which accumulate in degenerating cells are at first scattered; later they begin to collect near the nucleus, probably about the centriole. Later still, as the mass of granules increases, a clear centrosphere forms in the center of it, the granules surrounding its periphery. In xanthomata there are indications of a similar process, of which Figure 3 probably represents an intermediate stage before the appearance of the clear central area. Earlier stages were seen, but most of the cells were in the later stages and contained centrospheres of various sizes. Owing to the fact that the cells are orientated at various angles in the plane of the section, some cells, especially those in which the nucleus and centrosphere are parallel to the plane, show these relationships much better than the others. Although no definite structures can be made out in these spherical bodies, such as have been described by Dr. Lewis in tissue-culture cells, it is evident that we are dealing with giant centrospheres in these xanthomatous tumors. The details of the structure in the giant centrosphere cannot always be seen, as was shown by Lewis and Webster (1921) ⁴ in their study of giant cells in cultures from human lymph-nodes.

Seventeen xanthomatous tumors were studied with special reference to the presence or absence of these giant centrospheres. The latter were present in nine, or a little over 50% of the cases. Of these, seven also contained foam cells, which have been regarded as evidence of degeneration of tissue (C. Smith, 1912).⁵ One specimen (Surg. Path. No. 5151) contained foam cells but no giant centrospheres. The cells containing the pigment appeared very degenerate and it is possible that they had reached that late stage in the process in which the giant centrosphere is lost, as has been described by Dr. Lewis (1920) ³ in tissue-cultures. There were eight tumors in this series which had neither giant centrospheres nor foam cells. In the cells of these tumors the nuclei stained well, the growth seemed active and there was no evidence of degeneration.

It is well known that pigment occurs in several types of tumors and it was therefore thought advisable to examine some of these to ascertain if the giant centrosphere is present in all, or if it is a special characteristic of the xanthoma group. The study included 25 benign pigmented moles, 50 malignant pigmented tumors, 20 giant-cell tumors of bone containing blood pigment, and 10 bone sarcomata containing blood pigment.

In some of the cells of the moles it could be seen that the granules were grouped at one side or one end of the nucleus, about what was probably the centriole, but there was no evidence of a giant centrosphere.

In the pigmented tumors many of the tumor cells were undergoing degeneration, but although the pigment frequently appeared to be collected about the centriole, no giant centrospheres could be found. Occasionally, however, in cells that had taken in pigment liberated by the death of the tumor cells, these structures could be seen.

In the giant-cell tumors no giant centrospheres or foam cells could be seen. In every one of these cases the growth appeared to be active and healthy and resembled very closely that seen in the eight xanthomata which did not contain giant centrospheres or foam cells. The types of cells containing the pigment in the giant-cell tumors of bone appear to be identical with those that contain the pigment in the xanthomatous tumors, and one would therefore expect to find giant centrospheres in this class of tumors also, if the growth of the tumor became arrested and degeneration began. No evidence of either giant centrospheres or foam cells was found in the bone sarcomata.

Dr. W. H. Lewis, in his study of the giant centrosphere in tissue cultures, has shown that the so-called Plimmer body (cancer-cell inclusions, bird's-eye inclusions, etc.) and the giant centrosphere are identical. He points out that the presence of the giant centrosphere is evidence of disturbance in the metabolism of the cell and suggests that these bodies might be found in pathological conditions other than cancer. This prediction has been fulfilled in part by the discovery of the giant centrosphere in xanthomatous tumors.

It should be remembered that this discovery was purely an accident. If these cells had not contained pigment granules forming a corona about the giant centrosphere, this body would never have been observed in sections prepared according to our routine pathological technique. This suggests that, with more refined cytological methods, definite alterations in the cells might be found in a number of abnormal conditions which are now considered as functional because they show no definite pathological changes with the usual technique.

SUMMARY

Giant centrospheres were present in the pigment-containing cells of nine out of seventeen xanthomatous

tumors examined. They are probably to be regarded as evidence of degeneration.

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EXPLANATION OF FIGURES

FIG. 1.—Photomicrograph of a xanthoma (*Surg. Path. No. 3890*) which shows that a large number of the cells are filled with

pigment granules. Some of the cells appear as circumscribed black splotches in which no details can be distinguished. In some of the others can be seen the clear central area which is the giant centrosphere. One cell, which is seen to the right and a little below the center, shows both nucleus and giant centrosphere, the latter surrounded by a corona of granules. (X 480.)

FIG. 2.—Camera-lucida drawing of a cell containing pigment granules and a few adjacent nuclei from the same section as Fig. 1. The nucleus appears somewhat swollen and the outlines of the cytoplasm are indicated merely by the extent of the granules. The giant centrosphere occupies the center of the cell and displaces the nucleus to one side. The granules are collected about the centrosphere except where it comes in direct contact with the nucleus. (X 2600.)

FIG. 3.—Another cell, showing a younger stage in the accumulation of granules from the same section. The nucleus is swollen and the pigment granules are massed at one end of it, probably around a centriole. There is no evidence of a centrosphere. (X 2600.)

A NORMAL PREGNANCY FOLLOWING INSERTION OF THE OUTER HALF OF A FALLOPIAN TUBE INTO THE UTERINE CORNU

By THOMAS S. CULLEN

In the Johns Hopkins Hospital Bulletin for September, 1921, Dr. Henry N. Shaw reported the case of a patient upon whom I had operated on Oct. 6, 1919. Her right tube and ovary had been removed by another surgeon several years before.

She entered the Johns Hopkins Hospital with signs of a left tubal pregnancy. On opening the abdomen I found a left cornual pregnancy. This was removed but the outer half of the left tube was saved, and its inner end was sutured into the uterine cornu.

She subsequently became pregnant but lost her baby on March 11, 1921, at the end of the seventh month. There was a placenta prævia.

On June 26, 1922 I received the following letter from Andrews, S. C. "Mrs. J. R. L. asked me to write you that she gave birth to a seven and a half pound baby girl this morning at eight-thirty. Dr. D. S. Porter was with her, labor was perfectly normal, both mother and child are doing finely so far."

Those who use every effort to save the tubes and ovaries or part of them will from time to time be disappointed and may be forced to do a second operation. The results in this case, on the other hand, clearly demonstrate what may occasionally be accomplished by judicious conservatism.

AN OPERATION FOR THE TOTAL EXTIRPATION OF TUMORS IN THE CEREBELLO-PONTINE ANGLE. A PRELIMINARY REPORT

By DR. WALTER E. DANDY

The most frequent tumor in the cerebello-pontine angle is an encapsulated endothelioma arising from the leptomeninges. Rather loosely embedded in the lateral wall of the brain-stem, it is potentially a benign tumor by virtue of its encapsulation. Its complete removal offers a permanent cure to the afflicted individual but its extirpation has been attended by a mortality so high as to render such attempts inadvisable. In fact, the complete removal of such tumors with recovery has been regarded as impossible. As a result, a partial intracapsular enucleation has been the operation which has seemed to offer most to the patient, but it is obvious that such treatment of a potentially benign lesion is most unsatisfactory

to the patient, for the tumor must inevitably recur.

Five years ago, I completely removed such a growth from a patient who has since remained well. The growth was extirpated *in toto* by careful dissection around the tumor. Subsequently, two other tumors of the same type were similarly removed, but the results of such a method were too capricious and the mortality was too high.

Gradually a procedure has been evolved by which I believe these neoplasms can be successfully removed and with relative safety; the mortality should be little higher than from a subtotal removal of the contents of the tumor. The last two patients with cerebello-pontine tumors have been treated by this procedure and are well. The last

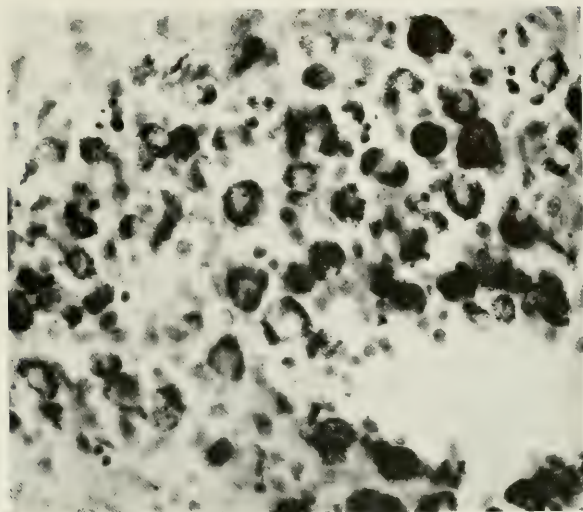


FIG. 1.



FIG. 2.

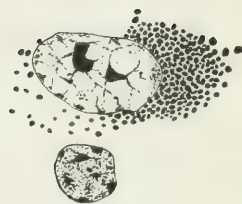


FIG. 3.

patient was a particularly bad risk because of a partial hemiplegia and hemianesthesia and inability to swallow. She quickly recovered from the operation. In one patient, the operation was performed in two stages; in the second in one stage. The latter method is far preferable because in the interim between stages the capsule becomes friable and more difficult to handle.

The purpose of this preliminary report is to present the salient features of the operative procedure. A bilateral suboccipital exposure of the cerebellum is performed with as much exposure of the affected angle as possible. The

interior of the growth is removed with a curette. Following this, the capsule is picked up with forceps and beginning at the upper and lower poles, carefully drawn away from the medulla, pons and mid-brain. The traction brings into view the several small veins and arteries crossing from the brain-stem to the tumor. These vessels are ligated individually with silver clips or fine silk ligatures and divided. Gradually, in this painstaking way, the whole tumor is delivered from its bed without bleeding, and without trauma to the brain-stem. The cranial nerves stretched by the tumor are automatically liberated as the capsule falls away from them.

NOTES ON NEW BOOKS

Human Embryology and Morphology. By ARTHUR KEITH. 4th Edition. Cloth, \$10.50. (New York, Longmans, Green & Co.: London: Edward Arnold, 1921.)

In the fourth edition of his *Human Embryology and Morphology*, Sir Arthur Keith has emphasized the importance of studying the development of the human fetus in relation to the general facts of comparative anatomy and evolutionary history. This unique approach is essentially characteristic of the broad conceptions of the author and is most important because morphological embryology of today must assist in unravelling the problems of phylogeny and growth, or remain relatively sterile. The presentation is clear and excellent throughout and furnishes a particularly appropriate introduction to the study of both human and comparative anatomy. The numerous views which have been advanced regarding many phases of embryology have evidently been searchingly analysed and no criticism can be made of those that the author accepts. But these views have been presented as final and in this the book fails to meet one of the most important requirements of a modern text-book. The student should be given every opportunity of deciding for himself in regard to opposing theories and should not be taught, too finally, any individual theory as established truth. With the addition of a few pages devoted to the presentation of both sides of certain disputed points, this book would have stood alone as embodying the two most important requirements of a text-book: a fundamental and inspiring viewpoint, and a presentation sufficiently broad to bring the student face to face with the problems of a growing science.

R. S. C.

Arterial Sclerosis. By LOUIS FAUGERES BISHOP. (London: Henry Frowde; Hodder & Stoughton, 1921.)

It is greatly to be deplored that, in the making of books of which there is no end, a more critical judgment should not be exercised in determining not only the value of the content but the length of many of the volumes that are offered to an ever credulous public with the stamp of approval conveyed by the publisher's imprint.

It is difficult to understand why there should be, apparently, so great a popular demand for a work of the type of this volume, as to necessitate a third printing, that adds nothing of constructive value to our knowledge of arteriosclerosis. The work is an extraordinary mixture of the personal experiences and opinions of the author together with many excerpts from the writings of others, the whole being combined in such a way as to lead often to much confusion in the interpretation of the text. In his preface the author states that "clinical medicine either in the hands of reputable or disreputable practitioners is always anticipating

the advance of knowledge," and further "the needs of physicians who have been trained in other specialties have been kept in mind and simplicity has been chosen rather than complexity."

In discussing the relationship of arteriosclerosis to blood pressure it is difficult to follow the author's apparent meaning. We disagree with the statement that "low blood pressure in arteriosclerosis may be regarded as an exception that always demands explanation." The following sentence quoted in full will illustrate our difficulty in interpreting the text—"The term blood pressure is often used where high blood pressure is meant. With light thrown on the meaning of the context this does not lead to confusion, though, of course, during life there is always pressure of blood in the vessels." (Chapter VI, p. 65, line 6 from top.)

We confess that we need light on "the context" and admit being led to confusion. We must, however, leave to his readers the ultimate decision as to the author's success in respect to the simplicity of his text.

The causes of arteriosclerosis discussed in a chapter of 16 pages contains not a single reference to the vast and fundamentally important literature upon the subject. The chapter entitled "The Natural History of Arteriosclerosis" following directly that devoted to the etiology, should be properly considered as a part of the former. Here again we have no reference to the literature and a delightful preliminary allusion to the life history of the oyster is followed by a most confused and inconsequential discussion of the subject. That the causes of arteriosclerosis are chemical and physical is a patent truism, but that the evolution of the disease process would in any large number of cases fall in with any such standard type as outlined on page 41, we seriously question. Great stress is laid upon high blood pressure apparently as evidenced by the systolic level, with but infrequent reference to the significance of the diastolic figures.

The chapter devoted to diet in arteriosclerosis and that upon the chemistry of the proteins should be read to be appreciated. The latter has at least the great merit of representing the statements of workers in this field from whose writings this chapter is largely constructed.

The essential purpose of the volume is apparently an exposition of the author's theories of the causal relationship between protein food intoxication and pathological cardiovascular changes, as well as his methods of therapeutic procedure in arteriosclerosis.

E. P. C.

Clinical Electrocardiography. By FREDERICK A. WILLIUS. Cloth, \$5.00. (Philadelphia and London, W. B. Saunders Company, 1922.)

This volume covers in an elementary way the fundamental principles of the physiology of the galvanometer in its adaptation to clinical use, together with a short chapter on the mathematics in-

volved, and brief statements of the mechanism concerned with the various cardiac arrhythmias.

During the past two years a number of similar works have appeared each dealing with the subject in a way peculiar to the individual author. It is, however, very doubtful that any genuinely new knowledge has been contributed through these sources or even that the debatable points involved have been in any sense cleared up.

In a work intended primarily as an introduction to the subject, one would wish that dogma might be less emphatic and that a more conservative judgment as to the conclusions arrived at from the interpretation of the galvanometric records might obtain.

Although it is quite correct to say that a negative "P" wave may indicate a shift in the pace-maker, such a statement unqualified may easily lead to confusion and convey a false impression. In Chapter X dealing with the abnormalities of the "P" wave, this earlier impression is in a measure corrected, though it is greatly to be desired that the text devoted to this phase of the subject be revised.

Paroxysmal tachycardia is dealt with in a little over a page and there is no adequate discussion of sinus tachycardia in relation to what the author calls paroxysmal sinus tachycardia and paroxysmal auricular tachycardia. Are we to accept a paroxysmal tachycardia of sinus origin as proven, and is the term paroxysmal auricular tachycardia to be used to connote the presence of an ectopic and heterogenetic rhythm with negative "P" waves in all three derivations? Unless we consent to this view it is, of course, incorrect to say that the "P" wave is always inverted in paroxysmal tachycardia auricular in origin (p. 79). In the references to the literature following the chapter devoted to the auricular tachycardias there is no reference to Bouveret's original article which even to-day should be read by every student interested in this subject.

In the discussion of auricular fibrillation, although the present interpretation of its mechanism based upon the work of Lewis and his co-workers is referred to, no allusion is made to the development of localised areas of block, which is the underlying fault leading to the condition of fibrillation and really distinguishing it from flutter, and no reference in this connection is made to the fundamental work of Meyer, Mines and Garrey.

In the light of our knowledge of the pathological physiology of flutter it is not correct to imply that the "P" wave is always inverted. While in some instances the "P" wave appears as a distinctly negative wave, in the vast majority of flutter records it can be read only as a diphasic curve (See Figs. 52 to 58), a fact which gives rise to the striking similarity seen from case to case, the only clinical condition of which this can be said to be true. The character of the "P" wave in the presence of a circus movement within the auricle must depend upon the relationship of the direction of the spread of the wave to the plane of the given derivation.

In discussing the subject of notched QRS complexes (p. 109) no allusion is made to the variation in these deformities which may occur under change of position and forced respiration seen so conspicuously in many instances, and depending upon the relationship of the plane of the derivation concerned to the direction of the axial spread of the excitation wave. To base sweeping conclusions upon nothing of this complex in any statistical relationship to the clinical diagnosis without first determining their variability, seems to us wholly unwarranted.

The chapter devoted to the abnormalities of the "T" wave is based upon a purely theoretical conception of the author without any adequate proof and cannot fail to confuse and mislead the reader who has no fundamental knowledge of the subject. Such an attempt to elucidate the mystery of the "T" wave can serve no genuinely useful purpose and the effort to classify clinical-pathological states on the basis of the negativity of the "T" wave in any combination of derivations is too absurd to merit discussion.

The history of the development of our knowledge following the introduction of the galvanometer as an instrument for the study of clinical cases is the story of a brilliant and extraordinary insight into more than one obscure corner of medical science; its use, however, as suggested by the figures from pages 162-182 of this volume can only give the uninitiated an utterly false conception of the usefulness of the instrument in clinical procedures.

E. P. C.

Textbook of Physiology. By RUSSELL BURTON-OPITZ. (Philadelphia and London: W. B. Saunders Co., 1920.)

The author's aim, as he states it, is to supply the medical student with a text-book in the subject of human physiology. As such, as far as it goes, the book accomplishes its end in a satisfactory way. Physiology covers a vast field and the making of a book of this kind is no light task. The number of subjects are too many to be fully understood by any one man. Hence, that the product of the author's labor should contain errors was inevitable. Some of these will gradually get corrected in the newer editions. But the fact that older authors do not succeed altogether in this leads us to be not too sanguine. To illustrate my point I need only refer to the figure on p. 147 of the present book, showing the distribution of electrotonus along a nerve. The figure is Pfliiger's down to the last detail of symbol and character of type, and the explanatory note below the figure is a free translation of Pfliiger's explanatory note buried in the text of his monograph. For these facts the reader need only turn to Figure 13, Plate V and p. 465 of the original. Yet not only does Burton-Opitz ascribe this diagram to American authorship but that master of text-books in Physiology, Professor Howell, chronically persists in doing likewise (see his 8th Edition). The error is clearly one of misapprehension or of oversight, and will persist, I presume, to the stroke of doom. In Burton-Opitz's book there is another figure—an original one—which clearly shows that here the author is describing something he does not well understand. The text describing the figure bears this out. I refer to the description of the principle, and application in physiology, of a thermopile, as given on page 97. The thing is a failure. When the author touches topics nearer his own fields of research one at once perceives the note of authority, and it is in these parts on the blood and circulation that the book has especial merit. Now this unevenness in text-book writing suggests that it is high time that physiology as a text for the student should no longer be written by one man. It has been suggested that, to avoid the difficulty of getting a dozen specialists to co-operate on one book, the subject could be satisfactorily covered by a dozen small treatises or special chapters separately printed. These could be revised and reprinted independently of each other and even by new authors as the older ones dropped the task. The convenience of handling and carrying the smaller volume need not be dwelt upon. Let us hope that the day of the cumbersome text-book is nearly ended.

C. D. S.

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THE EXCITATORY STATE*

By W. M. BAYLISS, M. A., D. Sc., F. R. S.

In this lecture I wish to call attention to certain phenomena associated with the excitatory state or state of activity. At the end of the previous lecture I mentioned the reversible change in protoplasm produced by an electrical shock, in which the liquid sol changes to a jelly with more or less solid properties. The use of the term "gel" has become somewhat indefinite and is sometimes used to mean any kind of precipitation of a colloid. The term "jelly" is sufficiently clear as typified by gelatin when cooled.

Similar changes have been described in cell-division, fertilization and so on, and the method of centrifuging the cell has proved very useful in work of this nature.

A point of interest is that if changes in consistency are indicative of activity, the protrusion of pseudopodia

is not active but passive, since the Brownian movements persist. This conclusion does not exclude active contraction elsewhere on the surface, however, and it seems difficult to account for the spherical form of leucocytes when stimulated otherwise than by a contraction of some kind over the whole organism. At the same time, all that the fact really implies is that the surface tension is equal all over.

The chief practical interest of the contractility of protoplasm is in its relation to the properties of the capillary blood vessels, which are essentially protoplasmic. This point will come up again in my next lecture.

Nerve and muscle (including the heart) are commonly regarded as the excitable tissues *par excellence*. But this is due to the fact that their response to stimulus is rapid and obvious. All living tissues are clearly excitable in so far as they respond by change of some

* Second lecture of the Herter Series. Delivered at Johns Hopkins University on March 8th, 1922.

kind to external influences. It is owing to the rapid character of the excitation process in the former tissues that so much investigation has been devoted to it in these cases. There are two aspects of muscular contraction to which I would call attention in connection with the problems of interfaces to which my first lecture was devoted. The first of these concerns the mode of production of the state of tension which the work of A. V. Hill has shown to be the primary fact. His researches have taught us the manner in which the magnitude of a contraction depends on the *length* of the fibres when the contraction begins. This means that the controlling factor is one of surface, not of volume; it is a question of surface energy, not of osmotic energy or of imbibition. The conclusion is confirmed by the results of experiments made to determine the temperature coefficient of the contractile stress. The most accurate and careful work has shown that this is negative; that is, the more powerful contraction is at the lower temperature. As previously mentioned, a surface tension phenomenon is implied thereby. The known production of lactic acid naturally suggests that the deposition of this acid or of its hydrogen ions on the muscle fibrils is the cause of the raised surface tension, as originally suggested by Haber and Klemensiewicz in their classical paper on "Interfacial forces." The presence of lactic acid as the cause of the contractile stress leads to a few remarks on the puzzling facts of "tonus" or "posture" in muscle. It has long been known that smooth muscle is able to enter into a state of shortening in which, as it appears, there is little or no expenditure of energy. This shortening is more or less reduced by rise of temperature; hence again, a surface tension effect is suggested. Since it must be supposed that in the usual form of contraction return to the normal length is associated with removal in some way of the lactic acid which causes the shortening, I have ventured to put forward elsewhere the view that the tonic state might be due to a prolonged remaining of the acid and that some influence may be required to set in motion the process which results in its removal. This would be something of the nature of an inhibitory process. Roaf has recently described experiments with a manganese dioxide electrode and finds that in a veratrinized muscle the acidity remains with the shortening, and that when decerebrate rigidity is reflexly inhibited, the acidity decreases. The latter fact shows that the muscle remains acid during the tonic contraction. But the behavior of the manganese electrode is not completely understood. The work of Sherrington on "plastic tonus" has directed attention to the presence in voluntary muscle of phenomena similar to those of smooth muscle. Various researches have shown the existence of certain features in common. I may mention my own work in which the heat production was found to be very small, and that of Roaf which indicates minimal pro-

duction of carbon dioxide. If the theory of the non-removal of lactic acid, as suggested above, be applied to voluntary muscle, it seems that it would be necessary to assume the existence in voluntary muscle of a distinct innervation process to set in action the relaxation, as in fact is the case with the inhibitory nerves of smooth muscle. This is no doubt a difficulty, but the theory of Bottazzi, according to which tonus is due to sarco-plasmic contraction, does not seem to me to offer a satisfactory solution. It is tempting to connect the sympathetic innervation of voluntary muscle with the tonic state, but it is remarkable how little direct evidence of this has been obtained. The sympathetic innervation remains a mystery.

The second aspect of the excitation process to which I would refer is the increased permeability of the cell membrane which is found to accompany it. The impermeability of the cell membrane in ordinary conditions to most of the ions with which it is in contact is, of course, the reason why living cells have so high an electrical resistance. When the membrane is made permeable, the resistance falls to a notable degree, as in Osterhout's experiments with sodium ions and with anaesthetics. These facts are the basis of Stewart's well known method of determining the proportion of plasma to corpuscles in blood.

I have already pointed out how impermeability to salts may be due to impermeability to one of the ions, the opposite ion being allowed free course, as far as permitted by electrostatic attraction. The result of such a state would be the presence on the outer surface of the cell of an electric charge of the sign of the permeating ion. Suppose that we place electrodes on two distant places of a muscle cell or a nerve fibre, and connect them to some instrument which indicates any potential difference between them, such as the string galvanometer. It has been known for many years that, provided that no injury has occurred, the two points are equipotential. Let us now stimulate one of these places. Our galvanometer shows by its deflection that the two electrodes are no longer equipotential, and further, that the stimulated region is electrically negative to that at rest. Now we know that we have changed the cell-membrane at the stimulated spot into a permeable one, so that the polarization rendered possible by the impermeability to one ion is abolished and the potential difference between the two sides of the membrane at the electrode on the point at rest is no longer opposed, while the direction of the current shows that the outside of the membrane is electropositive to the inside. We have, in other words, by making the excited spot permeable, made the interior of the cell as it were accessible to the leading off electrode. The ions which were arranged in two oppositely charged layers have been enabled to mix together and neutralize their effect. It is not, therefore, that excitation produces

a negative state, but the permeability brought about by it enables the polarized charge at the resting electrode to become evident. This is, briefly, the theory associated with the name of Bernstein, who pointed out that the nature of electrical conduction in solutions necessitated the view that the electrical changes observed in active tissues must be ionic in origin. In support of this conclusion, Bernstein measured the temperature coefficient of the electromotive force of some bioelectrical phenomena and found it to be of the very low value of that of the electrical conductivity of solutions of electrolytes. Some earlier views had associated it with a kind of chemical reaction, which would have had the usual high temperature coefficient of such phenomena. If follows naturally, from what has been said, that if a muscle is in a state of tonic contraction, an inhibition of this state under one electrode would make that spot appear to be positive to that still in contraction. It is of some interest to note that the complex electrical changes described by Orbeli as occurring in the ureter can be interpreted fairly satisfactorily as being due to waves of inhibition followed by waves of contraction similar to those in the intestine preceding and following a mass of food material and propelling it onward (the "law of the intestine" or "myenteric reflex").

My object in calling attention to the electrical phenomena in muscle and nerve is partly also to refer to some points connected with such phenomena as they occur in the heart and in the secreting glands.

But before passing to these, I would mention the work of Starling and his collaborators on the work of the heart muscle. At the beginning of this lecture it was pointed out how A. V. Hill had shown that the magnitude of the contractile tension developed in a muscle fibre depends on the length of the fibre at the initiation of the contraction. Now Starling has demonstrated that the same law holds for the heart muscle and that all the phenomena of the output of the heart in relation to arterial pressure, venous inflow and so on, are to be explained on the basis of the various degrees of filling of the ventricles at the moment of systole. The facts as applied to this organ are embodied in the "law of the heart."

The electrocardiogram is of course a complex case of the electrical change in a muscle fibre as described above, made complex by the fact of the excitation progressing as a wave along an elaborate system of conducting channels. I merely wish to express a doubt as to whether conclusions drawn from changes in the form of the curve have any great significance in relation to the actual working of the heart muscle. We must bear in mind that, owing to the potential difference led off being due to slight differences in the time of arrival of the excitation process at different spots, any state which alters even very little either the rate of the wave in one part

or its duration at one spot may exercise a profound influence on the actual form of the "ventricular complex." In the work which was done by Starling and myself on the dog's heart, we were able, by varying the temperature of the air used for artificial respiration, actually to invert the electrical change, without, so far as could be detected, producing any effect on the mechanical beat. The work of Mines and others showed further that, under certain conditions, the mechanical beat could be abolished, while the electrical change remained normal. This merely shows, however, that it is the excitation process that is responsible for the electrical change. Although, according to Einthoven, there may still be a very small contraction, detectable by sensitive methods, the independence in degree of magnitude of the electrical and the contractile change is clear. Again, the form of the "ventricular complex" is sometimes brought into relation with the rapid rate of conduction along the Purkinje tissue as compared with that in the contractile muscle. It has recently been shown that the heart of the chick embryo of 50 hours gives a similar electrocardiogram to that of the adult, although histological examination reveals a structure uniform throughout. Of course, these considerations do not apply to the use of the electrocardiogram in the investigation of block, extra-systole, auricular fibrillation and so on, but it appears to me that great caution is necessary in interpreting varieties of form of the ventricular complex itself.

In conclusion, I wish to refer briefly to the electrical phenomena of secretion, especially in the mammal. It is somewhat remarkable that practically no further work has been done in this region since that of Bradford and myself many years ago. It is now time for a renewed investigation in the light of modern electrical theory. But in the meantime it is worth while to examine how far the facts made out by Bradford and myself are to be explained on the lines of present views of the secretory process. In the first place, it appears that during the period of rest following activity of a secreting gland there is a steady accumulation in the cells of material which is to serve as the precursor of the contents of the secretion. This process is associated with the consumption of oxygen and comes to an end when a certain quantity has been produced. It seems to be controlled by mass action and to proceed automatically, without the intervention of nervous impulses. It is on the whole very similar to the restitution phase of muscular activity. Moreover, like the actual contractile phase of muscle, the act of secretion, when excited by nerve stimulation or by drugs, seems to be independent of oxygen. In the gland, what probably occurs is something like this: the material present in the cell, or some part of it, is split up into smaller molecules, so that the osmotic pressure in the cell rises. This causes attraction of water and momentary distension of the cell. But at the same time that

end of the cell in relation with the lumen of the alveolus and thus with the duct loses its semi-permeability, so that water, carrying with it solutes from the cell, escapes to the duct. Such a current flowing through the cell from the lymph space to the duct continues as long as osmotically active matter together with the permeable state of the one end of the cell remains. The process is similar to the current of water flowing through a tube of sugar solution if the one end is open, the other end closed by a semi-permeable membrane and immersed in water. It was found by Bradford and myself that the electrical change produced in the submaxillary gland of the dog on stimulation of the chorda tympani nerve was definitely associated with passage of water, although the state of knowledge at the time did not permit the more detailed explanation. Now, if the cell membrane of the secreting cell

has properties similar to that of the red blood corpuscles, namely, permeability to anions, impermeability to cations, the sign of the electrical difference between the fundus and the duct end of the cell in the excited state corresponds to what would be the case on the hypothesis outlined above. A similar change has recently been found by Anrep and Daly in the pancreas excited by secretin. When the sympathetic supply to the salivary gland is stimulated, there is a small electrical effect in the opposite direction to that of the chorda. The meaning of this requires elucidation. It is important that future work should be done, at all events in the first instance, on dogs, since it is only in these that the effects of the chorda tympani and of the sympathetic supply are clearly differentiated.

A STUDY OF FROZEN SECTIONS THROUGH A CADAVER SHOWING THE ANATOMICAL RELATIONS OF A LARGE UTERINE MYOMA

By J. WHITRIDGE WILLIAMS *

In May 1921, Professor Weed informed me that he had received from the Anatomy Board a female cadaver, which he thought might be pregnant. His records showed that the body had come from the morgue, and that the death certificate of the coroner stated that the woman was 26 years old and had died from natural causes.

Upon examination I found the partially frozen body of an apparently middle-aged colored woman, which presented no abnormalities except an abdominal tumor which reached to within three fingers of the xiphoid cartilage, but I could not determine whether it was a pregnant uterus or a tumor of some other kind. It was determined to freeze the cadaver thoroughly and then to section it in various planes in the hope that we might secure information concerning the relations of the pregnant uterus to the rest of the body and particularly to the pelvic floor.

After the body had been thoroughly frozen, the head and the legs were removed, and the trunk was bisected by a sagittal mesial section, which was so accurately made that it involved the pubic joint and the urethral canal. It was then found that instead of a pregnant uterus we had to deal with a lobular myoma, whose largest lobe distended the abdominal cavity, while a smaller lobe completely filled the pelvic cavity, with its lower pole protruding 2 cm. below the line joining the lower margin of the symphysis and the tip of the last sacral vertebra. In other words, the smaller lobe filled out the pelvic cavity and distended the pelvic floor just

as a child's head in the second stage of labor before it reaches the vulva.

Two other sagittal sections were made through the right half of the body—one being 3 cm. to the right of the mesial section, and the other 3 cm. to the right of the second. The left half of the body was divided by four oblique sections, which roughly corresponded to the oblique planes which Hodge employed so advantageously in studying the anatomy of the pelvis. The first of these extended through the promontory of the sacrum and the top of the symphysis, while the others were parallel to it and at varying distances below the plane of the superior strait.

Mr. Max Brœdel was kind enough to make tracings of the sagittal sections, from which he prepared the accompanying pen and ink drawings (Figures 1-3). The oblique sections were allowed to thaw in a solution of carbolic acid and formalin and were then photographed. After careful dissection the various landmarks were identified and have been indicated by Mr. Brœdel in the retouched photographs. I take this opportunity to express my sincere thanks for his artistic work, without which this communication would be of but little value, but I am especially indebted to him for his interest and advice during the study and dissection of the specimens.

The object of this communication is to demonstrate the topographical relations of a large myoma and to study the changes which it has produced in the pelvic structures, as well as to show the differences between the distention of the pelvic cavity by a myoma and that resulting from the head of a child at the time of labor. With this in view, I shall briefly describe the condi-

* Lantern demonstration before the American Gynecological Society, May 1922.

tions existing in sagittal sections 1 and 2, then I shall consider the degree of distension of the abdominal and pelvic cavities resulting from the presence of the tumor, as well as the displacement and compression of the abdominal contents. This will be followed by a description of the tumor itself, and the changes produced by it in the ovaries and the uterine ligaments. I shall then take up the consideration of the oblique sections through the left half of the body which illustrate the anatomical distortion produced by the presence of the tumor in the pelvic cavity, and finally I shall study the changes in the vascular supply of the pelvic viscera, as shown in figure 10.

Beginning with the study of the sagittal sections, figure 1, which represents the right side of the vertical mesial section through the trunk, shows that we have to deal with a large myoma whose upper extremity reaches to the level of the twelfth dorsal vertebra and which has compressed and displaced the abdominal and pelvic contents to an extraordinary degree. It is seen that the tumor consists essentially of two main portions—the larger one arising by a comparatively narrow pedicle from the anterior surface of the uterus near its fundus, while the smaller one, which distends the pelvic cavity, arises from the posterior surface of the uterus by a broad pedicle approximately 5 cm. in diameter.

Owing to the distension of the pelvic cavity by the smaller tumor and the upward traction exerted by the larger one, the uterus has been compressed against the symphysis pubis and has become greatly elongated and retroverted. From external os to fundus it measures 16 cm. in length. It is thinnest at the level of the upper margin of the symphysis and thickest just beneath the fundal region, measuring 1 and 2.5 cm. in the two locations respectively. The external os is sharply marked and its anterior lip occupies a somewhat lower level than the posterior, lying approximately 5 mm. above the lower margin of the symphysis. The bladder has been compressed between the uterus and the symphysis pubis in such a manner that its lower portion is represented by a mere slit, while its empty upper half is transformed into an abdominal organ which extends 5.5 cm. above the upper margin of the symphysis. The rectum has been so markedly compressed that only its ampulla and lowermost portion are visible. The intestines have in great part disappeared, and are represented by three small loops, which occupy an irregularly triangular space between the myomatous masses and the fundus of the uterus, and by several loops of transverse colon lying between the stomach and the apex of the tumor.

Anteriorly, in close contact with the lower pole of the abdominal myoma and the upper portion of the uterus, is a small triangular mass, which represents a section through the lower end of the omentum which was adherent to the tumor just to the left of the midline.

Before the section thawed, the space between the abdominal wall, the anterior surface of the uterus, and the myoma was occupied by discolored ice, slightly yellowish in hue, and which represented frozen ascitic fluid. Above the upper pole of the tumor it is seen that the abdominal organs are markedly compressed and that all trace of the gastro-intestinal tract has disappeared, except for the contracted stomach and four coils of transverse colon. The liver is in close contact with the under surface of the diaphragm, while the compressed contents of the thorax afford striking evidence of the compression to which the body cavities had been subjected.

Figure 2, which represents the right side of the same section 3 cm. from the midline, shows essentially similar conditions, except that it gives a better idea of the manner in which the larger tumor arises from the fundus, and shows the abundant vascular supply and its extension into the lower pole of the abdominal myoma. In this section the uterus appears thicker than in the midline, and the small vertical slit in its center represents a portion of the uterine cavity. It will be noted that the pelvic cavity is still completely occupied by the pelvic myoma, and in addition that two small myomatous nodules arise from the posterior wall of the uterus and project into the space not occupied by the larger tumor.

Section 3 illustrates the extent to which the body cavity has been distended and the abdominal contents displaced by the tumor. It was drawn after removal of the tumor, which was effected by cutting through its connections with the uterus and separating the various adhesions which fixed its lower pole to the pelvic floor. It is seen that except for the compressed rectum, which is visible only in front of the last three sacral vertebrae, and for the uterus and bladder, which lie just behind the symphysis pubis, the pelvic cavity is entirely empty. Indeed the only structures which can be recognized on casual inspection are the external iliac vessels, which, roughly speaking, indicate the boundary of the superior strait, and the somewhat dilated ureter which extends obliquely downward and forward beneath the peritoneum covering the lateral wall of the pelvis to join the bladder in front of and above the external os.

It is apparent that the major part of the uterus has become an abdominal organ, and, with the few coils of intestines posterior to it, forms a diaphragm which partially divides the body cavity into two portions; the upper of which was occupied by the abdominal and the lower by the pelvic myomatous mass. In the upper cavity it is seen that the tumor was in direct contact with the anterior and lateral abdominal walls, and had displaced the intestines from their usual location, compressing them into an irregular triangular packet which occupies only the posterior and lateral portion of the abdominal cavity. The omentum is greatly elongated and thinned, and extends downward from the lower margin

of the transverse colon for almost the entire length of the abdominal cavity to become eventually attached to the lower-most portion of the largest myomatous mass, just to the left of the mid-line, and to contribute to its nutrition by means of numerous large vessels which it contains.

Figures 8 and 9 represent the anterior and posterior aspects of the tumor, respectively. From these it is seen that the whole tumor, together with the excised upper portion of the uterus, forms an irregular mass measuring $37 \times 20 \times 15$ cm., and is made up of three main portions. The upper lobe, which in great part filled the abdominal cavity, is irregularly oval in shape, and measures $22 \times 21 \times 15$ cm. in its various dimensions. It arises from the neighborhood of the fundus by a relatively small pedicle. Below it and arising from the right side of the uterus just below the cornu is a pedunculated kidney-shaped myoma, $12 \times 16 \times 4.5$ cm. in its various diameters; while the third mass, which fills the pelvic cavity, arises from the upper third of the posterior wall of the right side of the uterus, with its pedicle extending only slightly to the left of the midline. This lobe measures $15 \times 14 \times 11$ cm., being greatest in width at its upper portion, while its smaller rounded end was adherent to and distended the pelvic floor. At the junction of its upper and middle thirds a distinct furrow is visible, which has resulted from pressure upon the linea inominata and external iliac vessels. Above this depression the tumor expands on either side, probably as a result of the presence of smaller myomatous nodules within it.

Figure 9, which represents the posterior aspect of the tumor, gives a good idea of the pressure which had been exerted by it, as well as of the compression to which it had been subjected by the various parts. In the upper third of the midline of the pelvic nodule a distinct depression is visible, which corresponds to the location of the promontory of the sacrum; while below it there is a slight convexity which corresponds to the lateral concavity of the hollow of the sacrum. Slightly to the right of this concavity is a broad vertical shallow groove, which has apparently resulted from pressure upon the rectum. Moreover, on the posterior surface of the larger tumor there is a vertical shallow depression which apparently has been produced by pressure against the second, third and fourth lumbar vertebrae, while to the left of this is a narrower, but somewhat deeper, depression indicating the extent to which the aorta had been compressed.

The lower tumor had contracted numerous adhesions with the peritoneum lining the base of the pelvic cavity, which had to be severed before the tumor could be removed. Many of the adhesions were of considerable thickness and contained numerous small vessels, and at first led to some misapprehension when it was attempted to ascertain the relations of the pelvic fascia.

Upon inspecting figure 8 it is seen that the uterus had been deflected somewhat to the right, and had been divided by the sagittal mesial section in such a way that its vertical axis lay just median to the insertion of the left round ligament and the uterine end of the left tube. Moreover, it is apparent that its increased length was in great part due to hypertrophic elongation of the supravaginal portion of the cervix, while the cavity of its body was little, if at all, increased in size.

Owing to the fact that the pelvic cavity was completely filled by the lower myomatous mass and that the uterus had been drawn far up into the abdominal cavity by the traction exerted upon it by the larger tumor, the tubes and ovaries have become abdominal organs, while the anatomical relations of the round ligaments have been profoundly altered. The median ends of both tubes are approximately normal. The lateral portion of the left tube is adherent to the external surface of the left ovary and is so compressed as to be recognized with difficulty. Its fimbriated end, however, is free and can be identified by a long ovarian fimbria, which is attached to the external surface of the upper pole of the corresponding ovary. The right tube, except for its abnormal location, is apparently normal and at its lateral end is in contact with the flattened-out ovary. This measures $6.5 \times 4.5 \times 0.5$ in its various diameters, and presents a convex external and a concave internal surface, as the result of its compression between the tumor and the abdominal wall.

The ovarian ligaments are normal in size and except for their upward course present the usual relations. But for the fact that they are situated far above the pelvic brim, the infundibulo-pelvic ligaments, likewise, show nothing unusual except the presence of unusually large vessels. Upon laying back the peritoneum, the ovarian vessels were exposed, when it was found that the arteries were extremely tortuous and convoluted and measured 5 mm. in diameter. The veins were made up of numerous branches which had eventually united to form a single trunk. On the left side this occurred at the level of the fourth lumbar vertebra, above which the single vessel presented a diameter of 12 mm.

Owing to the distortion produced by the tumor the relations of the round ligaments on the two sides vary greatly. As shown in Fig. 8, the right ligament presents essentially normal relations, while the left has undergone great distortion. The former is comparatively short, and consists of a uterine portion 4 cm. long, and a terminal portion of about the same length, which occupies the right inguinal canal and terminates in the upper part of the labium majus. The latter, on the other hand, is greatly elongated and measures 22 cm. in length. Instead of extending upward, forward and outward to reach the internal ring, it extends downward and backward from its uterine origin to penetrate the perito-

neum at a point 10 cm. posterior to the midline and 4.5 cm. above the level of the superior strait. It then turns and pursues a downward and forward course beneath the peritoneum, to disappear in the mons veneris, 4 cm. to the left of the midline.

As has already been indicated, the uterus has in great part become an abdominal organ, so that 11 cm. of its length lies above the level of the superior strait. Upon dissecting the broad ligament and the adjacent structures, it is found that the peritoneum forming the uterovesical sac can be separated from the anterior surface of the uterus to within 4.5 cm. of its fundus, above which further separation is impossible. As figure 1 shows that this is considerably above the apex of the bladder, it gives an idea of the extent to which further distention of that viscus could occur.

Our description thus far applies in great part to the general topography of the tumor and to the effect which it had exerted upon the economy of the woman. On the other hand, information of more particular gynecological and obstetrical interest may be gained from the study of the sections through the left side of the body which were made parallel to the superior strait.

Fig. 4 represents a retouched photograph showing the relations at the superior strait. The section passes through the top of the symphysis and the cartilage covering the promontory of the sacrum, and extends upward and backward to the skin of the back in such a way as to involve the body of the fifth, the arch of the fourth and the spinous process of the third lumbar vertebra. It corresponds to Hodge's first oblique plane and shows clearly the extent to which the soft parts encroach upon the pelvic inlet.

The pelvic cavity is completely filled by the myomatous mass, which has pushed the uterus so far forward that it is separated from the posterior surface of the symphysis only by the compressed slit-like bladder. It is interesting to note how large a portion of the ilio-psoas muscle occupies the iliac fossa, as well as the fact that the external iliac vein follows practically the contour of the linea innominata, but is in contact only with its anterior and posterior ends. It should be noted that the triangular space just lateral to the body of the first sacral vertebra is occupied by the hypogastric artery and vein, the ureter and by the obturator nerve. Anteriorly, it is seen that the uterine body is directly in contact with the bladder and so compressed that it does not exceed 1 cm. in thickness. The broad ligament extending from its margin is greatly thinned out and lies in contact with the anterior and lateral walls of the pelvis, instead of extending transversely across the middle of the pelvis, and dividing it into an anterior and posterior pocket. It should further be noted that the anterior face of the broad ligament lies in contact with the pelvic fascia, that the double fold of peritoneum which should

normally be present has disappeared; while the peritoneum covering its posterior face constitutes the peritoneal lining of the anterior portion of the pelvis. In other words, the myoma occupies the immensely distended pouch of Douglas, and has displaced and compressed everything in front of it.

Fig. 5 represents a section parallel to, and two centimeters below, the superior strait. It bisects the symphysis pubis and the body of the first sacral vertebra, while posteriorly it involves the arch of the fifth and the spinous process of the fourth lumbar vertebra. It will be noted that the bony ring of the pelvis is here complete, being formed posteriorly by the body of the sacrum, laterally by the iliac, and anteriorly by the pubic portions of the innominate bone.

The sacro-iliac joint is well shown, and it is apparent that in this instance the sacrum does not form the keystone of an arch, but would prolapse into the pelvic cavity under the influence of the body weight were it not held in place by strong ligamentous structures. It is likewise apparent that the myoma completely fills out the pelvic cavity and has led to intense compression of the uterus, which at this level is represented by a cross-section of the flattened cervix. The broad ligament is much thinner than in the preceding section and lies in direct contact with the thinned-out lateral ligament of the bladder, which extends outward from the margin of that viscus.

Probably the most striking feature in this section is the presence of a small segment of the ilio-psoas muscle, which has descended beneath the level of the linea innominata and actually encroaches upon the pelvic cavity. The distended ureter in its course towards the bladder has come to occupy the middle of the lateral pelvic wall and is separated by the pelvic cavity only by peritoneum and sub-peritoneal fascia. The various arteries and veins are well shown, and passing down over the anterior surface of the lateral mass of the sacrum is the lumbosacral trunk. In this section it is apparent that, while the ureter lies median to the pelvic fascia, the main vessels in the posterior segment of the pelvis are enclosed within sheaths derived from it. It is important to note that in this section there is as yet no sign of the obturator internus, the pyriformis or the levator ani muscles.

Fig. 6 represents the upper surface of the third parallel section and lies 2.5 cm. posteriorly and 3 cm. anteriorly below the section just described. This plane bisects the second sacral vertebra, while its anterior end terminates in the external genitalia half a centimetre below the lower margin of the symphysis. For practical purposes it corresponds with Hodge's second parallel plane and with Veit's main plane of the pelvis, and would be identical with the latter did its ends involve the lower margin of the first sacral vertebra and symphysis pubis respectively. Here it will be noted that the bony wall of

the pelvis is no longer complete, its anterior portion being represented only by the descending ramus of the pubis, to whose left lie the erectile structures just beneath the pubic arch; while to the right is the obturator foramen, which is covered internally by the median end of the obturator internus muscle.

The sacro-iliac joint is well shown, and it will again be noticed that the sacrum represents an inverted keystone of an arch, and that only a fraction of its thickness takes part in the formation of the joint. Laterally, where the innominate bone is sharply constricted and is devoid of cancellous tissue, is a plate of compact bone which corresponds to the apex of the sacrosciatic notch. In this section it is again evident that the pelvic cavity is completely occupied by the myomatous mass. Anteriorly, the uterus has disappeared and has been replaced by the vagina. Attention is directed to the fact that the thinned-out broad ligament of the previous sections has given place to its thick vascular base and to the perivaginal tissues. The former lies internal to the lateral vesical ligament, and gradually fades away as the middle portion of the pelvic wall is approached. Furthermore, it should be noticed that the uterine artery has receded from the midline, and, roughly speaking, occupies a position opposite the lateral margin of the obturator foramen.

Anteriorly and laterally, the obturator internus muscle is seen as a thin structure varying from 2 to 5 mm. in thickness, extending from the ischio-pubic ramus to the ischial spine, and giving no suggestion of the dimensions it will assume in the next succeeding section. In the posterior quadrant of the pelvic cavity, and lying in front of the sacro-iliac joint, the sacral plexus may be seen approaching the sacrosciatic notch. It lies external to the pelvic fascia and is separated from the bone only by the superior gluteal vessels, and its location affords a ready explanation for at least some of the pain, which may be associated with relaxation of that joint. In the extreme anterior and median portion of the section the labium majus and minus are visible, while posterior to them lie the various erectile tissues associated with the clitoris.

Fig. 7 is very instructive and gives a conception of the topography of the pelvis which cannot be gained by the ordinary methods of study. This section, which is parallel to the preceding one, lies 2.6 and 2.8 cm. beneath its posterior and anterior ends respectively. Posteriorly it extends through the tip of the third sacral vertebra, while its anterior portion involves the external genitalia and is obliquely traversed by the urethra just beneath its upper surface. The tumor still occupies the greater part of the pelvic cavity, but the striking features of the section are afforded by the relations of the pelvic muscles, the sciatic nerve and the base of the broad ligament. At this level it is seen that the obturator internus has become transformed from the thin structure visible

in the previous section into a thick-bellied muscle, which extends from the descending pubic ramus to the ischial spine while external to the body of the ischium its strong tendon can be seen on its way to its point of attachment in the depression at the back of the trochanter.

The pyramidalis muscle presents a striking picture and appears as a surprisingly large structure, which arises from the greater part of the anterior surface of the sacrum, and passes through and almost completely fills the sacro-sciatic notch on its way to its attachment to the femur. Internal to the obturator internus can be seen an oblique section through the levator ani muscle, which at this level varies between 3 and 4 mm. in thickness. It can be traced upward for about half a centimetre into the preceding section, where its insertion to the posterior surface of the symphysis and ischio-pubic ramus, to the white line, and to the ischial spine can be located. In this section the muscle is covered on either surface by fascia, while above the two layers fuse into the single layer of pelvic fascia above the white line. Under normal conditions in this location the peritoneum covering the internal layer of fascia over this muscle should line the entire pelvic cavity, but in the specimen under consideration a thick layer of abundantly vascularized pelvic connective tissue is interpolated between it and the pelvic cavity. In other words, the anterior segment of the pelvic cavity has become obliterated, while Douglas' cul-de-sac has become greatly expanded.

In this section, likewise, we see the sciatic nerve in oblique section, which affords a vivid picture of its magnitude and teaches us how readily its median end can be subjected to pressure by an object distending the pelvic cavity. Just anterior to the nerve and almost in contact with the ischial spine can be seen the internal pudic vessels. On inspecting the anterior portion of the section, the vagina is represented by two slits, which are directed obliquely backward and are separated by a thick layer of highly vascularized tissue. This is the result of an oblique section through a fold of its mucosa, and by no means indicates that the canal is double. Posteriorly the sacro-iliac joint is again seen, and once more it shows that the union at the sacro-iliac joint is such that, if the sacrum were not held in place by strong ligamentous structures, it would inevitably collapse into the pelvic cavity.

This section represents almost the base of the pelvic cavity; for in the next one, which lies 3 cm. beneath it and extends through the tip of the fifth sacral vertebra, all that is left is a shallow oval area about 5.5 cm. in diameter, and 5 mm. in depth, lying immediately over the coccygeal vertebrae. Posteriorly, this depression is occupied by peri-rectal tissue which is abundantly supplied with vessels, while anteriorly and laterally the thinned-out margins of the levator ani can be distin-

guished, whose fibers fuse with and disappear into those of the sphincter ani. The greater portion of this last section, which is not figured, is made up of an oblique section through the gluteal muscle, outside of which comes a layer of subcutaneous fat which varies from 2 to 5 cm. in thickness.

Upon dissecting the lower surface of the fourth section from below, the ischiorectal fossa can be opened up as a large potential cavity bounded internally by the levator ani and its fascia, and externally by the layer of fascia covering the obturator internus, the pyriformis and the gluteal muscles, and reaching its apex at the level of the white line.

Thus far, we have studied the relations of the tumor to the pelvic cavity and to the structures within it. Incidentally, we have mentioned the enlargement of the ovarian vessels and those of the broad ligament, and we shall now study the changes which have occurred in the vessels supplying the pelvic organs. These are best studied in figure 10, which represents the lateral wall of the pelvic cavity after the removal of the tumor, as seen in a sagittal section through the right side of the body, 3 cm. from the median plane. The striking features of this section are the relations of the ureter and of the pelvic vessels. The former is dilated to several times its usual calibre and constitutes a hydro-ureter. Posteriorly the vessels present essentially normal conditions, while anteriorly they show numerous deviations from the usual relations which have resulted from the presence of the tumor and the consequent need for an increased vascular supply.

The median surface of the section involves the lowermost end of the common iliac vein just before it divides into the external and internal iliac veins. On the other hand, the point of division of the artery lay higher up and median to the plane of the section, so that the external and internal iliac arteries appear as separate vessels.

The external iliac artery and vein need not concern us here, as they have already been considered in connection with Fig. 4, where the latter formed the outer boundary of the superior strait. The internal iliac or hypogastric vessels are, however, of great importance. Shortly after entering the pelvic cavity the hypogastric artery divides into three main branches. The outer one continues forward, upward and eventually inward toward the umbilicus, as the obliterated hypogastric artery. The median branch is the uterine artery which is unusually elongated and enlarged. After passing beneath the ureter, it follows the pelvic wall as a vessel with many convolutions almost to the symphysis pubis where it enters the base of the broad ligament to supply it and the uterus, instead of entering it at about the middle of the pelvis. The posterior division is the largest of the three, and is much larger than under usual conditions.

After traversing the posterior portion of the pelvis for several centimeters, it divides into the inferior gluteal and the middle and inferior hemorrhoidal arteries.

Thus it is seen that except for a considerable increase in the size of all vessels and the striking elongation and tortuosity of the uterine artery, the arterial supply of the pelvis differs but little from normal. On the other hand, the venous supply has become extraordinarily expanded, and the section clearly shows that the lateral pelvic wall is covered by a number of large trunks, each of which approximates the external iliac vein in size, and which extend from the anterior portion of the pelvic cavity in order to carry away the blood from the enlarged uterus and its appendages. It is evident that they here form a plexus, which has attained immense proportions and covers the entire middle third of the lateral and anterior pelvic walls, and which drains the vesical and utero-vaginal regions. Posteriorly, just in front of the pyriformis muscle, the lumen of an immense vein is visible which has probably resulted from the union of the gluteal and hypogastric veins.

This picture was obtained by turning back the peritoneum and the subperitoneal fascia, and shows that all of the vessels in question were surrounded by their own fascial sheaths and lay median to the main pelvic fascia, so that, in their anterior portions at least, they were separated from the levator ani muscle by that structure. In the lower part of the section a small segment of the rectum is seen, whose anterior and upper wall has been deflected in order to expose the vessels beneath it. This section likewise affords an excellent picture of the relation of the muscles of the pelvis. Anteriorly, the obturator externus and obturator internus muscles are seen upon the two aspects of the obturator fascia, respectively. Median to and in close contact with the obturator internus, the anterior half of the levator ani muscle is seen, which with its posterior portion forms a diaphragm concave above and convex below, which extends from the horizontal ramus of the pubis to the ischial spine. In this section the muscle scarcely exceeds 1 mm. in thickness and is considerably thinner than in Fig. 7; while in its anterior portion, immediately adjacent to the bone, it is almost fascial in character. Overlapping its posterior extremity and lying external to it, a section through the coccygeal muscle is visible, which is only a fraction as extensive as the levator. Still further posterior and higher up, a section of the pyriformis is seen in the shape of a large triangular muscular mass lying between the anterior and inferior end of the sacral ala and the upper and anterior surface of the gluteus maximus muscle. In front of the pyriformis are the lower two, and above it and in contact with the anterior surface of the sacrum are the upper two, of the four spinal roots of the sacral plexus; while at the uppermost por-

tion of the anterior surface of the sacrum a section of the lumbo-sacral trunk is visible. This is in marked contrast with the appearance of the sacral plexus as shown in Fig. 7, and illustrates the conditions more median to its point of origin. In front of the pyriformis muscle the internal pudic artery can be seen making its way toward the ischial spine on its course to the external genitalia.

This completes in a cursory way the description of what the various sections show, and it only remains to recapitulate in a few words the changes brought about by the presence of the tumor and to compare the behavior of the pelvic floor with what would have resulted had the pelvis been distended by a foetal head late in labor.

In the first place, the specimen gives an extraordinarily accurate idea of the extent of the abdominal and pelvic distension which may be produced by the presence of a large myoma. Owing to the fact that the nodule, which almost completely filled the pelvic cavity, arose from the posterior surface of the uterus, the distention has occurred altogether posteriorly to that organ, with the result that it together with the bladder has been compressed against the anterior pelvic wall.—In other words, the distention has occurred entirely within the pouch of Douglas. This has resulted in a remarkable displacement of the broad ligaments, so that they are in contact with the anterior and lateral wall of the pelvic cavity instead of dividing it into an anterior and posterior segment; the former has become obliterated and the latter immensely distended. Owing to the forward displacement of the uterus and of the median ends of the broad ligaments, great elongation of the uterine arteries was necessary. This apparently has occurred to an even greater extent than was essential, as is shown by the fact that instead of entering the base of the broad ligament shortly after they have been crossed by the ureters, the arteries extend forward almost to the symphysis pubis before so doing, and at the same time have become unusually convoluted. Moreover, even though myomata are in general very poorly vascularized, the demands for the nutrition of the tumor have necessitated an increase in the blood supply, as is shown by the

unusual hypertrophy of the pelvic veins, which has attained a degree encountered only in full-term pregnancy.

Upon comparing the changes in the pelvic floor in this instance with those occurring during normal pregnancy, several very important points of difference should be noted. In the first place, when the pelvic cavity is occupied by the foetal head late in the second stage of labor, the distension proceeds from a central point, with the result that the cervical canal together with the base of the broad ligament becomes expanded outwardly in all directions, to an equal extent, so that while the anterior and posterior peritoneal pouches are temporarily obliterated, no such change occurs as was noted in this instance. In it the pelvic tumor lay behind the uterus, and consequently, as it increased in size, the posterior pouch became immensely distended, while the anterior pouch was obliterated by the cervix being pressed firmly against the symphysis pubis. At the same time the broad ligaments were flattened out against the anterior and lateral portion of the pelvic wall, instead of involving its posterior segment as well. The distension has likewise led to comparable changes in the relations of the pelvic fascia and the structures beneath it, which, instead of being pushed centrifugally outwards from a common center, have become distended only posterior to the uterus, with the result that the levator ani muscle forms a continuous but thin pelvic diaphragm, instead of being perforated and pushed downward and outward to form the lower-most portion of the birth canal.

Finally, particular attention is directed to the unusual picture of the anatomical relations which have been obtained by the employment of oblique sections through the pelvis parallel to the superior strait, and which indicate the advantages which might well follow the use of similar sections in the study of the dislocation of the pelvic floor during labor, as well as in the study of the part played by the bases of the broad ligaments in the maintenance of the normal position of the uterus in the non-pregnant woman.

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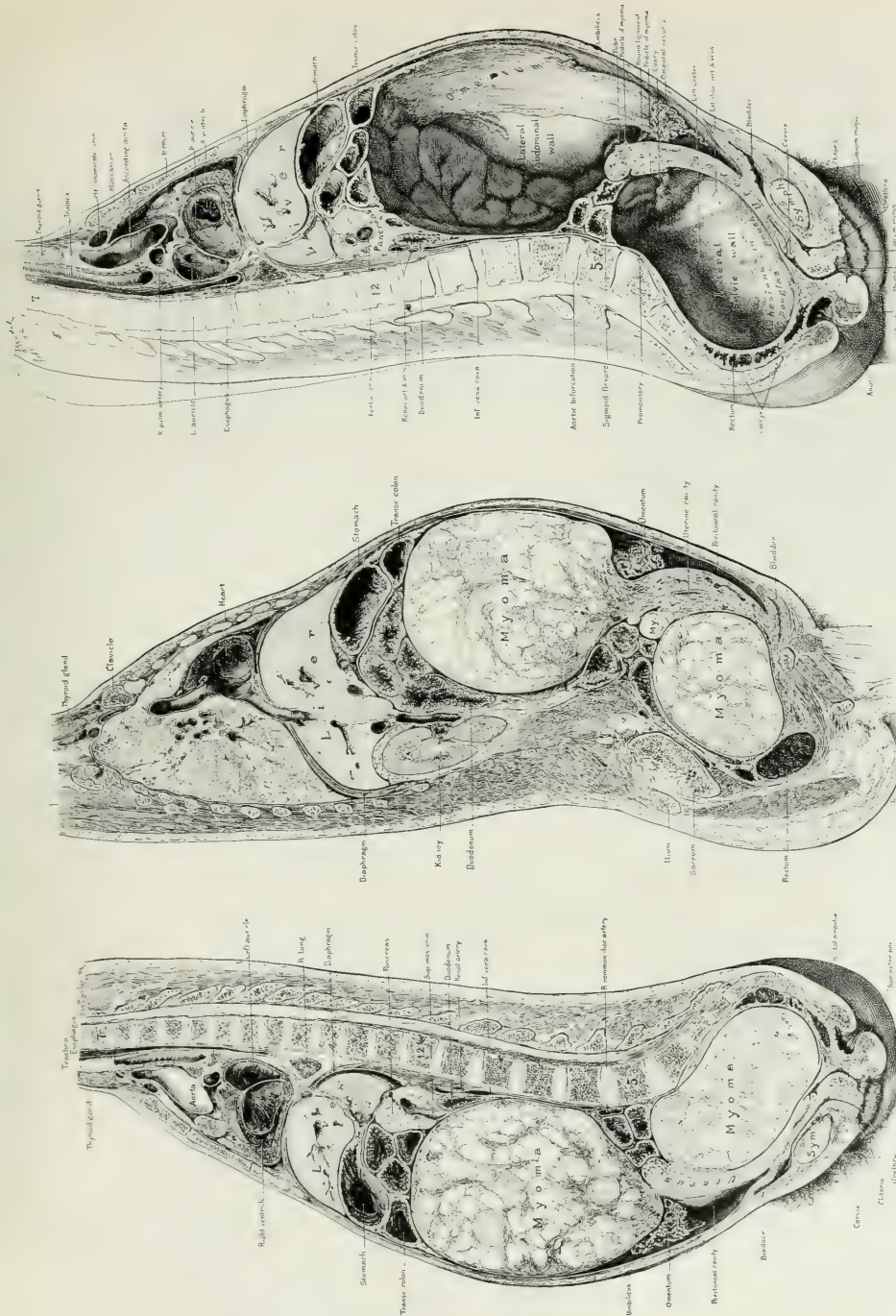


FIG. 1.—Sagittal mesial section, showing relations of tumor and displacement of viscera. $\times 1/4$.

FIG. 2.—Reverse side of figure 1, 3 cm. to the right of midline, showing relations of tumor, and particularly the connection between the abdominal nodule with the fundus uteri. $\times 1/4$.

FIG. 3.—Sagittal mesial section, showing left half of the body after removal of tumor, illustrating the distension of the abdominal cavity and the displacement of the pelvic viscera. Note the compression of intestines and the elongation of omentum. $\times 1/4$.

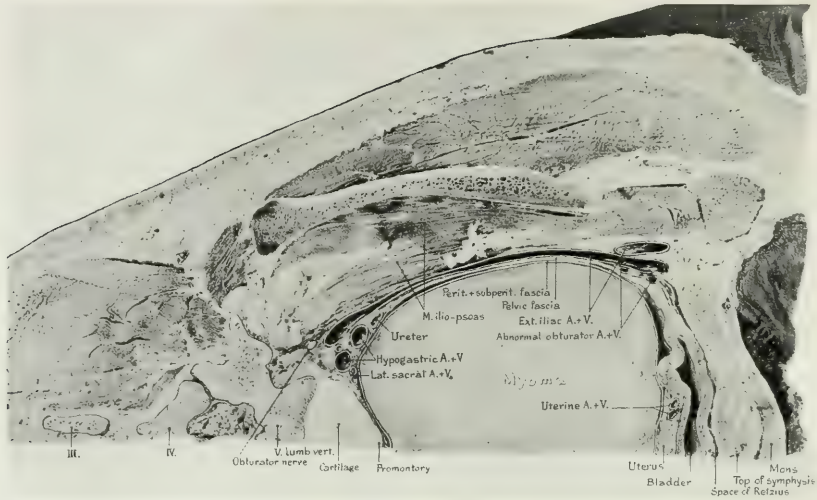


FIG. 4.—Oblique section through left half of body, showing relations at plane of superior strait. $\times 1/2$.

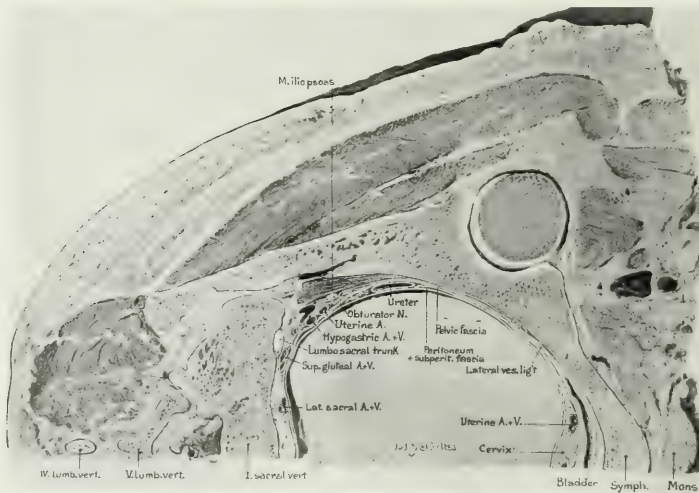


FIG. 5.—Oblique section parallel to and 2 cm. below that shown in figure 4. To demonstrate absence of pelvic muscles in this area. $\times 1/2$.

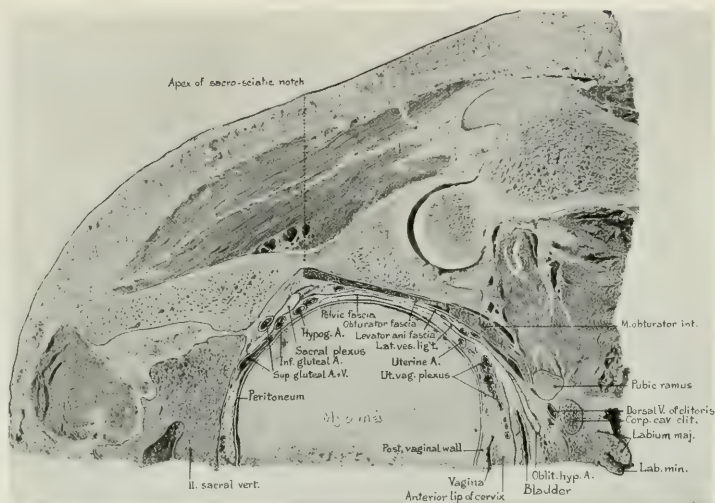


FIG. 6.—Oblique section through left half of body, parallel to and 5 cm. below that shown in figure 4. (Hodge's second parallel plane). $\times 1/2$.

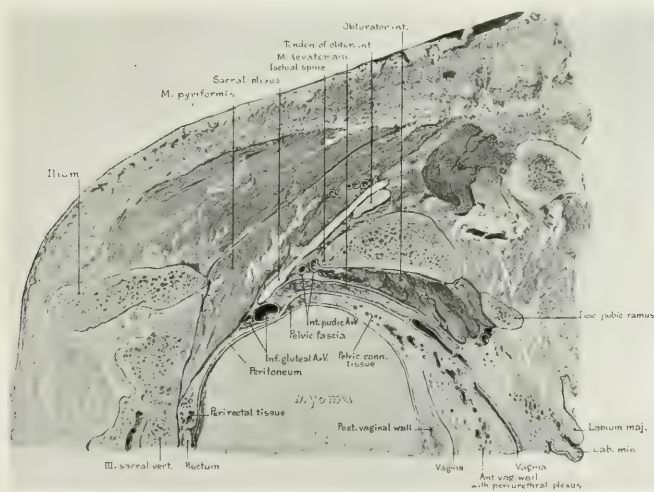


FIG. 7.—Oblique section parallel to and 2.5 cm. below that shown in figure 6, extending through the tip of the third sacral vertebra. To illustrate the extent of the pelvic musculature. $\times 1/2$.

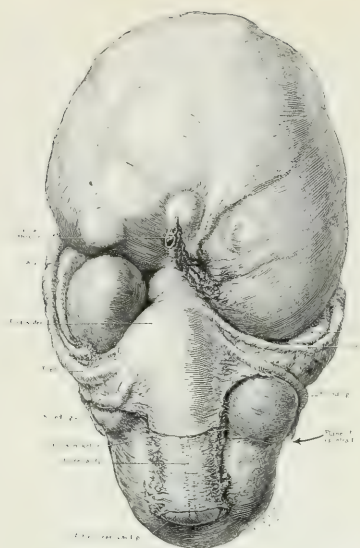


FIG. 8.—Anterior view of tumor showing its relations to the uterus, round ligaments and tubes. $\times 1/3$.

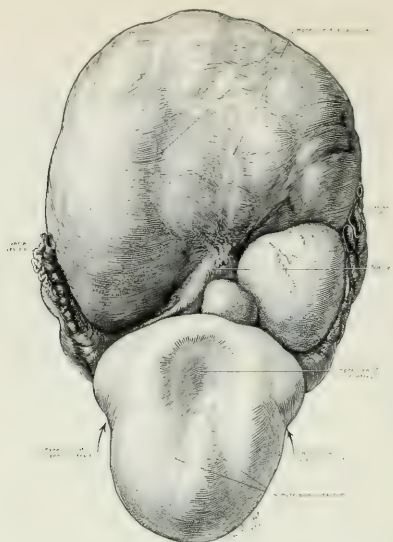


FIG. 9.—Posterior view of tumor showing indentations due to pressure exerted by the margins of the superior strait and vertebral column. $\times 1/3$.

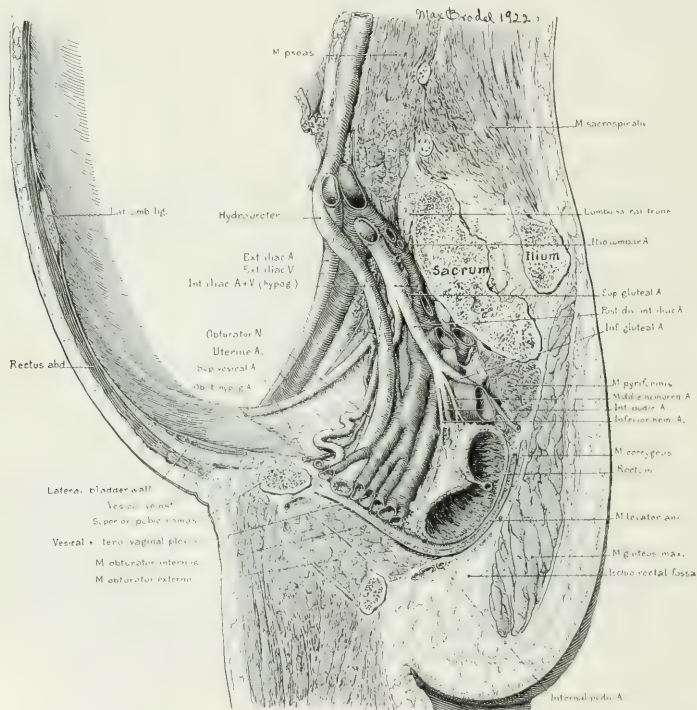


FIG. 10.—Sagittal section through right side of body, 3 cm. from midline, adjacent to figure 2, showing relations of blood vessels, ureter and pelvic musculature. $\times 1/2$.

THE CHANGES IN THE PARA-OCULAR GLANDS WHICH FOLLOW THE ADMINISTRATION OF DIETS LOW IN FAT-SOLUBLE A; WITH NOTES OF THE EFFECT OF THE SAME DIETS ON THE SALIVARY GLANDS AND THE MUCOSA OF THE LARYNX AND TRACHEA

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In a previous paper¹ I showed that the primary changes which occur in the eyes of rats on diets deficient in fat-soluble A are xerosis conjunctivæ, and xerosis corneæ (xerophthalmia). These changes are identical with those of xerosis in human eyes which are believed to result from dryness of the tissue. The ulceration and destruction of the cornea which follow xerosis corneæ and conjunctivæ (keratomalacia) are due to the secondary infection of the cornea by micro-organisms. Since the primary change in these tissues is due to dessication, it would seem logical to look for the cause of the trouble in the organs whose secretions under normal conditions serve to moisten the conjunctival sac. The secretion of the lacrimal gland—the tears—is chiefly concerned with the moistening and cleansing of the surface of the anterior portion of the eye. Beside the secretion of the lacrimal gland the conjunctival sac receives that of the Meibomian (tarsal) glands in the lids. The Harderian gland in the orbit also pours its secretion into the conjunctiva, and the mucous cells in the membrane itself contribute also to the conjunctival fluid. All these secretions are mingled in the conjunctival sac, and the resultant mixture moistens and protects the eyeball. The anterior part of the eye is kept clean by this secretion in which there is a more or less constant current, running toward the nose, which serves to wash foreign material through the nasolacrimal duct. This fluid also checks the multiplication of micro-organisms, since the tears possess some bactericidal power. How effective this mechanism is in removing bacteria from the conjunctival sac may be judged by the fact that bacteria, after being sprayed into the conjunctival sac, may be recovered from the nose in five minutes.² The purpose of this paper is to report the changes which occurred in the above-mentioned glands from animals in various stages of experimentally induced xerophthalmia. Moreover, I have also attempted to correlate the changes in these para-ocular glands with those in the salivary glands, and the changes in the conjunctiva with those in the mucosa of the larynx and trachea.

Lacrimal gland.—The most striking change in the tissue of this gland in animals suffering from xerophthalmia is in shrinkage of the secretory cells of the acini. These cells become very much shrunken. They no longer show secretory zones. The nuclei take on irregular shapes, occupy the central part of the cytoplasm, which is very small and stains very poorly. The individual acini of the gland become so small that it is impossible to distinguish them with the low power of the microscope, and the gland appears to be a simple mass of nuclei. It is not to be expected that a gland in this condition can produce tears. In a certain number of animals the cytoplasm of the gland cells is occupied by very large vacuoles to such an extent that the bioplasm itself has almost disappeared. The nuclei of such cells are small, centrally located and deeply stained. Frozen sections stained with Sudan III and sections from material which has been fixed in Fleming's solution show that some of these apparent vacuoles are in reality droplets of fat. Glands such as I have described are those which are most severely affected, but even in those that are most nearly normal, the cells show no evidence of pre-secretory or secretory activity; they are clear and small, and do not stain well. The poorly stained nucleus is situated in the basal portion of the cell, surrounded by small vacuoles in the cytoplasm, whose central portion consists only of a fine network. Although it is possible that such cells may produce some secretion it is quite certain that the production would be in very much diminished quantity.

The above findings indicate that the lacrimal glands of animals affected with xerophthalmia produce little, if any, secretion; in any case it is difficult to believe that any secretion which they might produce could be normal. It is quite certain that some of these glands have ceased secreting entirely.

Meibomian gland.—Sections of the lids of the eyes of animals suffering from xerophthalmia very often show cystic dilatation of the ducts of the Meibomian glands. These cysts are filled with fat—the secretion of the gland cells. The epithelium of the margin of the lid shows

evidence of a very marked xerotic process, and the same change is found in the epithelium of the duct. It is very likely that this xerosis may play a part in the occlusion of the duct and the formation of the retention cysts.

Harderian gland.—The lumina of the acini of this gland may be either very much dilated and empty, or narrowed by swelling of the secreting cells. Frozen sections of this gland show a remarkable diminution of the fat content of the cells and the lumen. The connective tissue about the gland acini is very often densely infiltrated with round cells.

The mucous cells of the conjunctiva are all entirely destroyed in the course of the xerotic process.

It is quite certain from these findings that the entire secretory apparatus of the eyes of these rats is in a state of dysfunction, that is, the secretion from these glands is either very much diminished or entirely lacking. The changes in the lacrimal gland are the most important in the pathological picture of this disease, and in fact the changes in this gland would seem to be the cause of the lesions in the cornea and conjunctiva. The diminution or the lack of the secretion of the lacrimal gland would explain the dryness of the eyeball as well as the xerosis of the cornea and the conjunctiva. The failure of the lacrimal secretion would, moreover, explain the increase in number of the organisms found in the conjunctival sac.

Salivary glands.—Having found very evident changes in the lacrimal gland during the course of xerophthalmia, it seemed logical to study the salivary glands which both in nerve supply and in structure resemble the lacrimal glands very closely. I therefore made a comparative study of these glands in rats affected with xerophthalmia. The glands of normal and of xerophthalmic animals were fixed in 10% formalin, or in Fleming's solution, embedded in celloidin, or cut in frozen sections and stained with hematoxylin-eosin, iron hematoxylin, safranin, and Sudan III.

The submaxillary and parotid glands of the rat are serous; the sublingual is a mucous gland. In many rats with xerophthalmia either all or some of these glands are either not secreting at all or secreting very little. The secreting cells become much shrunken. The acini are very small and show no traces of secretion. The epithelium of the intra-lobular ducts is shrunken and the cells are irregular in size.

The epithelium of the principal ducts of these glands often shows a remarkable degree of cornification and desquamation of the thickened superficial cells, so that the lumen of the duct becomes narrow and is often occluded. These changes resemble the xerotic changes in the eye. In two cases the occlusion of the ducts of the sublingual gland was followed by the dilatation of the duct and the gradual atrophy of the gland cells. Dilatation of the small ducts occurs frequently in the parotid

gland. Coincident with the xerotic changes and those in the parenchymal cells, the ducts are invaded by bacteria and small abscesses are formed in the gland. In other words, the findings in the salivary gland are of a nature similar to those in the lacrimal gland, and the duct epithelium undergoes a change which is identical with those which occur in the conjunctival epithelium. The reason for the infection of the salivary glands in these animals probably explains also the parotitis which sometimes complicates typhoid fever. The glands are directly infected from a dry mouth which contains an abnormally large number of micro-organisms.

The other secretory organs, such as the liver, pancreas, bowel, kidneys, thyroid, showed no remarkable change in sections except in one case. In this animal the cytoplasm of a small number of cells of the pancreas was very much vacuolated. The reproductive glands of rats on a xerophthalmia-producing diet do not function. Reproduction in these animals almost never occurs. Wason³ has reported fibrosis of the testes in two animals which she examined after they had been on a xerophthalmia-producing regimen.

Clinically and at autopsy, the mucous membrane of the mouths of these rats was very dry. The animals thus afflicted in the early stage of the disease sneeze and cough violently, but later the cough subsides spontaneously and after a period of dyspnea the animals die. Nevertheless, histological sections of the mucosa showed no more than the normal cornification of the epithelium. On the other hand, the mucous membrane of the larynx and trachea showed xerotic changes. The normal epithelium of the larynx and trachea is ciliated columnar in type, except that of the vocal cords, which are covered with stratified epithelium. The xerotic changes first appear in the epithelium of the larynx. The outer layer of cells in this region becomes cornified and stains deeply with eosin. The cells lose their nuclei, and become much flattened. In the second layer typical granules of kerato-hyaline appear. The epithelium becomes much thickened and is often infiltrated with pus cells. Gradually this xerotic change involves the tracheal mucosa. Xerosis then becomes complicated by an inflammatory process which often involves the bronchi and lungs and may terminate in a bronchopneumonia. Such a pneumonia was responsible for the death of most of the animals which were not sacrificed. The mucous cells in the mucous membrane are completely destroyed. Those which are embedded in the submucosa show evidence that their secretion is either diminished or that their secretory power is entirely destroyed. It is easily seen from the foregoing description how closely these changes in the mucosa of the larynx and trachea resemble those in the cornea and conjunctiva. If the same conditions obtain in the larynx and trachea as in the conjunctival sac, the bacterial content of the air passages should be much increased

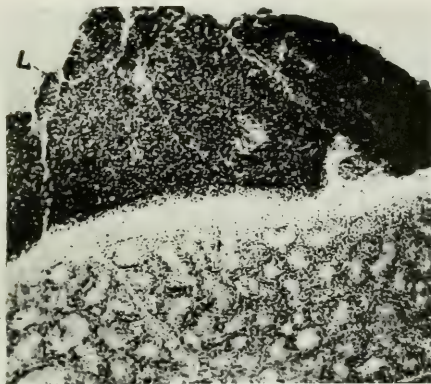


FIG. 1

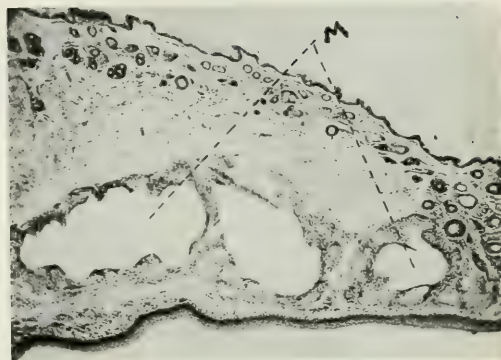


FIG. 2

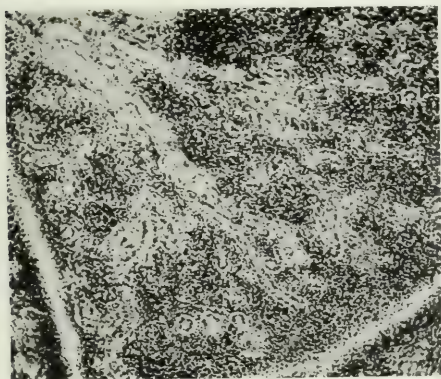


FIG. 3

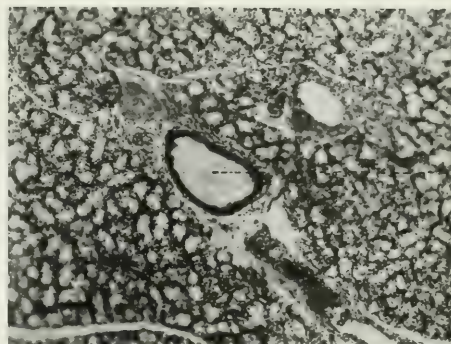


FIG. 4

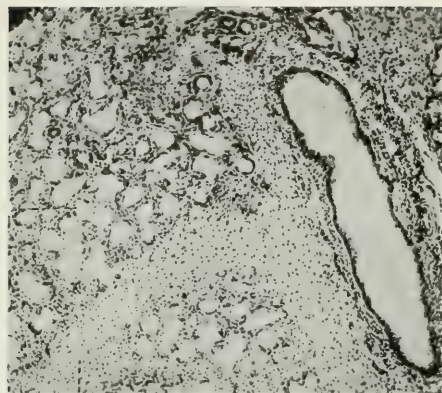


FIG. 5

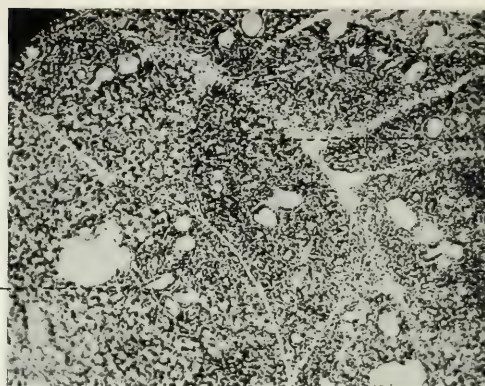


FIG. 6

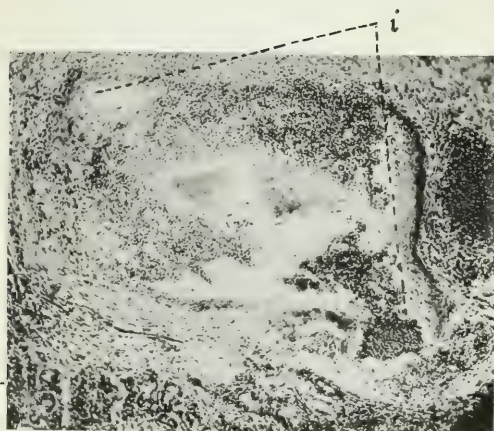


FIG. 7

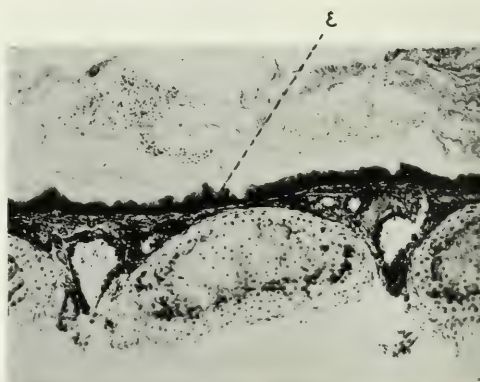


FIG. 8

because of the drying of the mucosa and loss of motility of the cilia of the epithelium. At any rate just as xerosis of the cornea is followed by secondary infection and the formation of corneal ulcers, so xerosis of the larynx and trachea is followed by inflammatory changes which eventually spread and involve the bronchi in the lungs.

The secretion of tears is, as a rule, either scanty or absent in children suffering from xerophthalmia or keratomalacia even during crying. These children are, as a rule, very hoarse and the voice is never clear. As is the case in rats with experimental xerophthalmia, the larynx in children is implicated in the pathological process, and like the rats the children usually die from bronchopneumonia. The pathological changes in rats explain the hoarse voice, the cough, bronchopneumonia and bronchitis, and the absence of tears and saliva which are seen in human cases of this disease.

If two per cent of cod-liver oil be added to the diet of xerophthalmic rats, the conjunctival sac becomes moistened, the number of bacteria becomes diminished, the xerotic change in the epithelium disappears. Coincidentally with the healing of the xerosis of the eye the lacrimal and salivary glands become normal histologically and they show histological evidence of secretion.

The results of these studies make it seem probable that the effect of cod-liver oil, or fat-soluble A, is not exerted directly upon the tissues of the eyes and the lids. More probably the vitamin acts, either directly or indirectly, on certain of the secreting glands in the body to insure their normal function, which is held in abeyance if the animal is deprived of his optimal intake of the vitamin. From this point of view it is necessary to consider the

clinical picture of xerophthalmia as a series of secondary symptoms which are indicative of hypo-function of a certain part of the secretory apparatus of the organism. In other words, xerophthalmia, as it is ordinarily seen, is the manifestation of a specific glandular lesion. It is one of a chain of symptoms, the other links of which are hoarseness, cough, lack of saliva, sterility, and general malnutrition.

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DESCRIPTION OF FIGURES

FIG. 1.—Shows the shrinkage of the cells of the lacrimal gland (L) and the Harderian gland (H).

FIG. 2.—The retention cyst formed as the result of the occlusion of the duct of the Meibomian gland (M) of lid.

FIG. 3.—To show the shrinkage of the secreting cells of the submaxillary gland.

FIG. 4.—This figure shows the cornification (C) of the epithelium of the principal duct of the sublingual gland.

FIG. 5.—Atrophy of the sublingual gland cells and extrusion of mucus into the interstitial connective tissue caused by the occlusion of the principal duct.

FIG. 6.—Cornification (C) of the intra-lobular ducts of the parotid gland and the partial shrinkage of the secreting cells (SC).

FIG. 7.—Infection through the duct of the submaxillary gland (I). The epithelium of the duct is very much thickened and the glandular cells have become atrophic (C).

FIG. 8.—This figure shows xerosis of the epithelium of the trachea and the desquamation of the cornified and thickened epithelium (E).

NOTE ON A MODIFICATION OF THE CHROMAFFIN REACTION, WITH OBSERVATIONS ON THE OCCURRENCE OF ABDOMINAL CHROMAFFIN BODIES IN MAMMALS

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That the cells of the medulla of the adrenal glands give a brownish reaction when fixed in a solution containing a salt of chromic acid was first observed by Henle ('65). This observation was extended by Stilling ('90), who showed that in the dog, cat, and rabbit there are groups of cells associated with the sympathetic nervous system, that react similarly with chromium salts. These he called "chromophil cells." Kohn ('03) abundantly confirmed Stilling's observations, showing, in the cat and rabbit particularly, that "chromaffin cells," as he designated them, occur frequently in association with the sympathetic nervous system, either as microscopic clusters of cells embedded in the ganglia, or as

bodies of macroscopic size located in the sympathetic plexuses. These groups of cells he termed "paraganglia." He directed attention to a conspicuous strip of chromaffin cells situated ventral to the abdominal aorta and superior to the inferior mesenteric artery, which was present in all of the mammals he studied and which he termed the "paraganglion aorticum abdominale." Finally, Zuckerkandl ('01) discovered a group of paired bodies in the region of the inferior mesenteric artery in human fetuses and new-borns which gave the chromophil reaction. These are usually referred to as the "organs of Zuckerkandl."

The methods employed by all subsequent observers for demonstrating these bodies have been those recommended by Stilling and Kohn. The retroperitoneal tissue of the animal is exposed and cotton, saturated with 3.5 per cent potassium bichromate, is placed in the abdomen for from 6 to 12 hours. The retroperitoneal tissue is then excised and washed for several hours in running water and then examined for the presence of chromaffin bodies, which, when present, should stand out as brownish strands or patches against a lighter colored background. A modification of this method consists in immediately excising the tissue suspected of containing chromaffin bodies and immersing it in 3.5 per cent potassium bichromate for the same length of time, followed by washing. It is advisable to transfer the tissue finally to glycerine in order still further to bring out the chromaffin bodies.

When subsequent microscopical examination of the tissue is desired, it is recommended that the chromaffin reaction be performed by fixation in formalin-bichromate (formaldehyde 40 per cent, 10 c.c.; potassium bichromate, 3.5 per cent, 90 c.c.). In all of these methods it is of course desirable that the tissue be fixed as soon after death as possible, for in the course of 6 to 18 hours post mortem the reaction no longer occurs in the chromaffin cells.

That the technique is sufficient to demonstrate the chromaffin bodies in man, dog, cat and rabbit is evidenced by the observations of Stilling, Kohn and Zuckerkandl and the subsequent ones of Swale Vincent ('12) and Kahn ('12). Although the principal bodies are readily distinguishable to the naked eye, in these animals, small scattered bits of chromaffin tissue cannot always be differentiated from clotted or extravasated blood without microscopic examination. Furthermore, Swale Vincent ('12) has been unable to discover any chromophil bodies in some animals, viz., monkey, pig, guinea-pig, rat, gopher, and squirrel. Kahn ('12) also reports the absence of macroscopic chromaffin bodies in four adult monkeys examined by him. One might assume that all of these animals lack macroscopically demonstrable chromaffin bodies. On the other hand, a suspicion might be aroused that the chromaffin reaction, as at present performed, is inadequate for their demonstration. The author proposed therefore to modify the technique, as heretofore used, and to investigate a number of animals for the presence or absence of chromaffin bodies. In the present paper this modified technique is described, together with results obtained in the few mammals which it was possible to secure for this purpose.

TECHNIQUE

The tissue to be studied was excised from the freshly killed animal, washed as free of blood as possible in running tap-water and placed in a large volume of fixative consisting of 90 parts of 3.5 per cent potassium bichromate and 1 part of 40 per cent formaldehyde (Kohn's

fluid). The tissue was not allowed to remain in this fixative over 3 hours, at the end of which time the chromaffin reaction was complete; it was then rinsed in tap-water and transferred for further fixation to 10 per cent formalin for 24 hours, after which it was washed thoroughly in running water and transferred successively to 60 and 70 per cent alcohol. The tissue was bleached by allowing it to stand for from 6 to 24 hours in sunlight in several changes of 70 per cent alcohol, prepared by adding 20 parts of hydrogen peroxide U. S. P. and 10 parts of water to 70 parts of alcohol. When completely bleached, the tissues, other than the chromaffin bodies, appear perfectly white. The chromaffin bodies stand out conspicuously (depending on the species of animal), as lemon-yellow to dark brown bands, threads, dots, or patches against a white background. The material may now be stored in 80 per cent alcohol, or portions of it embedded and cut. Excellent permanent gross preparations were obtained by dehydrating the material and clearing it in benzol and methyl salicylate according to the Spalteholz method. In these transparent preparations the chromaffin tissue stands out as a series of brown bodies whose position in reference to the blood-vessels is readily visible.

The observations of the earlier investigators concerning the size, shape, number, color, and distribution of the abdominal chromaffin bodies in the dog, cat, rabbit and in man have been amply confirmed by this technique. Further, the opossum, guinea-pig, squirrel, rat, and monkey were examined and, with the exception of the rat, abdominal chromaffin tissue was demonstrated macroscopically in all of them.

Opossum (two adult females).—On bleaching the retroperitoneal tissues, from 8 to 10 lemon-yellow bodies, about 1 mm. in diameter, became visible, scattered irregularly in a plexus of nerves extending from the level of the adrenal glands to the bifurcation of the aorta. On section these bodies proved to be ganglia in which were embedded groups of from 10 to 30 cells whose cytoplasm contained dark brown stained material (Fig. 1).

Guinea-pig (three adult males and three adult females).—In every instance, on examining the bleached retroperitoneal tissue, a conspicuous orange-colored stellate mass, measuring 2 mm. in diameter, was observed in the midline in the celiac plexus, immediately below the superior mesenteric artery. On section this proved to be a mass of cells possessing a distinct capsule and exhibiting a characteristic chromaffin reaction (Fig. 2). No chromaffin bodies were demonstrable in the gross elsewhere in the abdomen.

Squirrel (one adult male grey squirrel).—After bleaching, an orange-colored mass, measuring 2 mm. in diameter and irregularly stellate in outline, became visible in the midline in the celiac plexus immediately below the superior mesenteric artery. On section this proved to be

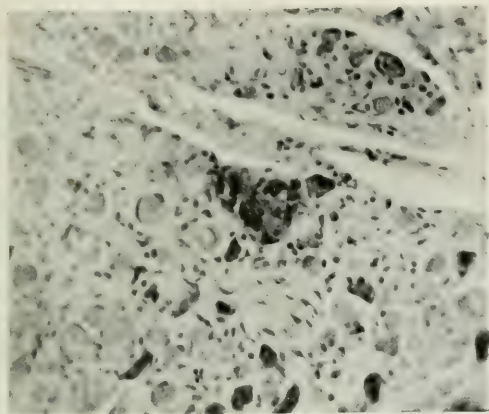


FIG. 1

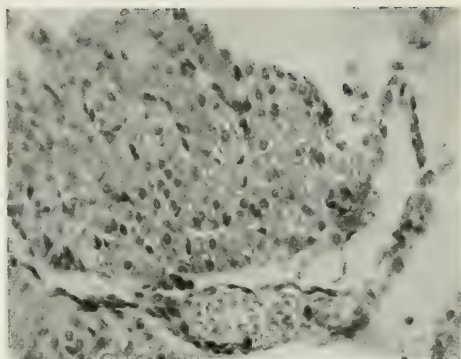


FIG. 2

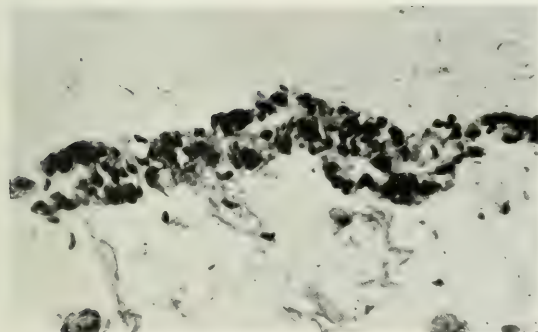


FIG. 3

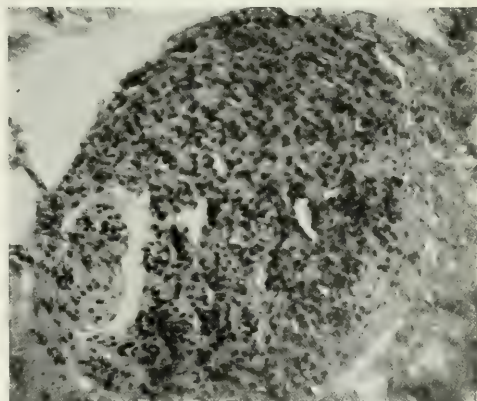


FIG. 4

Plate.—Wislocki, G. B. A Modification of the Chromaffin Reaction, with Observation on the Occurrence of Abdominal Chromaffin Bodies in Mammals.



a characteristic group of chromaffin cells (Fig. 3). No chromaffin tissue was visible to the unaided eye elsewhere in the abdomen.

Rat (three adult males and three adult females).—After bleaching the retroperitoneal tissue, the material was carefully examined with the naked eye and with the aid of a lense, but no masses of chromaffin tissue could be discovered.

Monkey (one adult female macaque).—After bleaching the retroperitoneal tissue, a series of small yellow bodies appeared, extending in a broken chain from the region of the superior to the inferior mesenteric arteries. The largest of these measured 2 mm. in length. On section these bodies proved to be typical masses of chromaffin cells (Fig. 4).

SUMMARY

A modification of the technique for demonstrating chromaffin tissue is described, whereby the presence of chromaffin bodies of macroscopic size can be demonstrated in the retroperitoneal tissue of the opossum, squirrel, guinea-pig, and monkey.

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DESCRIPTION OF PLATE

- Fig. 1. Sympathetic ganglia from the aortic plexus of an opossum, showing a group of chromaffin cells. (× 400.)
 Fig. 2. Chromaffin body from the coeliac plexus of a guinea-pig. (× 320.)
 Fig. 3. Chromaffin body from the coeliac plexus of a squirrel. (× 290.)
 Fig. 4. Chromaffin body from the aortic plexus of a macaque. (× 200.)

ON THE CARDIAC COMPLICATIONS OF GONORRHOEA

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The old concept of gonorrhœa as of a local process in which complications arise largely through direct extension, or as a result of secondary infections, has long since been abandoned. The chills, prostration, leucocytosis, anæmia, which are associated with the common complications of gonorrhœa, epididymitis, cystitis, pyelitis, synovitis, arthritis, the fever and more or less severe general symptoms which may attend the onset of an apparently uncomplicated specific urethritis, are often, in their severity, quite out of proportion to the apparent gravity of the focal process. They are indeed manifestations which might lead us to suspect the existence of a septicæmia. And since the communication of Hewes in 1894, from various sources there has been accumulated abundant evidence that gonococci are often present not only in the focal lesions but also in the circulating blood. Furthermore, it has been shown that 'tis not only in those cases with grave general manifestations that septicæmia may be demonstrated. Gonococci have been found in the circulation in instances of mild continued fever in apparently uncomplicated gonorrhœa (Thayer, 1905).

The occurrence of endocarditis as a complication of gonorrhœa and its direct dependence on the diplococcus of Neisser has now been demonstrated by many observers.

Gonorrhœa, then, is an infection, focal at first, which spreads not only by direct extension, but also and not infrequently, through the blood stream—a general septicæmia associated with a variety of metastases, notably arthritis, synovitis, myositis and not infrequently endo-my- or peri-carditis.

How frequently and under what guise do we meet with the cardiac complications of gonorrhœa?

This is a very difficult question to answer, and consultation with the literature leaves one often in doubt because of the insufficiency of the criteria on which diagnoses are made. The one source of accurate information, at the outset, is the study of those fatal cases which have been confirmed by necropsy.

There is a considerable literature dating from the sixties and before, concerning gonorrhœal peri- and endocarditis. At the outset these complications were generally regarded as secondary infections. Gradually, since the discovery of the gonococcus, and especially, since 1889, evidence has been accumulated which has demonstrated that these complications are in the beginning generally pure gonorrhœal infections.

The consecutive steps by which this conclusion was reached were:

1. The demonstration of gonococci, microscopically in the focal lesions at necropsy.

2. The association of this observation with the negative results of cultures taken *intra vitam* on ordinary media.
3. The obtaining of positive cultures, *intra vitam*, and the demonstration of the organisms, microscopically and by culture, in the blood or valvular vegetations at the necropsy.
4. Reproduction of an acute urethritis in man by inoculation with gonococci obtained at the necropsy from the affected valves.

In 1899, the writer had already collected thirty-two instances of well-described ulcerative and vegetative endocarditis associated with gonococcal infection, with post-mortem records, and fifteen in which the purely gonococcal nature of the infection might fairly be accepted.

The occurrence in the medical service of the Johns Hopkins Hospital, in the summer of 1920, of a case of gonorrhœal endocarditis of the pulmonary valves has turned our attention again to a subject in which we have been interested for years. Since then we have observed two additional cases. We have, accordingly, reconsidered our experience with gonorrhœal endocarditis at the Johns Hopkins Hospital during the last thirty-three years.

At the outset it may not be uninteresting to record briefly the three recent cases above referred to.

CASE I. Gonorrhœa. Ulcerative and vegetative endocarditis of pulmonary valves—Cultures from the circulating blood, negative—Gonococci demonstrated in the valvular thrombi at necropsy and cultivated from the kidney.

E. R., (Med., 44082; Path., 6319), a coloured boy of 19, between ten and eleven weeks before his death was seized with a stitch in his right side associated with a palpable friction rub. This was followed by fever, chills, sweats, cough, progressive weakness, increasing anæmia (R.b.c., 4,224,000; hb., 80%;—R.b.c., 2,528,000; hb., 28%), high leucocytosis (20,000-28,000). There was moderate cardiac enlargement with some dullness in the first and second left spaces, and a loud to and fro murmur in the pulmonary area, well transmitted throughout the chest; no essential change in the aortic sounds. Irregular, intermittent fever (99.4°-103.8°); albuminuria; pus and blood in the urinary sediment.

Gram-negative diplococci, morphologically gonococci, were demonstrated in the prostatic fluid. Cultures from the blood were repeatedly negative.

The patient failed rather rapidly. The transverse diameter of the heart increased in extent and a systolic murmur became audible in the tricuspid area. Severe diarrhœa set in with blood-stained stools. Eight days later, ten weeks after onset, the patient died.

The course of events seemed to be clear. Acute ulcerative endocarditis of the pulmonic valve; pulmonary embolism with infarction and pleurisy causing the first subjective symptoms; intermittent fever, chills, progressive anæmia, gonorrhœal prostaticitis, cystitis, pyelitis, nephritis, terminal colitis, death.

The necropsy wholly supported our clinical diagnosis. The heart was not remarkably enlarged. The aortic, mitral and tricuspid valves showed no abnormalities. There was an extensive ulcerative endocarditis with large, irregular, nodular vegetations on the pulmonic valve which showed no evidence of a pre-existing stenosis. There were two infarctions in the right lung, one older, in the right lower lobe, and partially organized, associated with pleural adhesions which occupied a position in the

right axilla corresponding to the area in which the patient had complained of pain at the outset; the other, fresher, in the lower lobe posteriorly. There was acute urethritis, hæmorrhagic cystitis, and pyelitis. The kidneys were large, swollen, cloudy and dotted with small hæmorrhages.

Smears from the thrombi on the valves showed numerous Gram-negative, biscuit-shaped diplococci. Cultures from the kidneys revealed gonococci; blood and vegetations, negative. There was no evidence of a pre-existing pulmonic stenosis or indeed of any pre-existing valvular disease.

CASE II. Acute vegetative and ulcerative aortitis with aneurysm formation—Cultures from circulating blood negative—Gonococci demonstrated in the focal lesions at necropsy.

A. S. (Med., 45333; Path., 6545), a coloured man of 28, with an indefinite history of an attack, five years previously, of pain about his heart followed by occasional sensations of palpitation on exertion, was seized, 8 weeks before death, with an arthritis in the right ankle and shoulder, and transient pains in the other joints. The symptoms of arthritis soon disappeared but a cough set in, so distressing as to interfere with sleep. The sputa were scanty, but on the 21st of March, three and a half weeks before death, were blood-stained. There was progressive weakness, sweating at night, and five weeks after the onset of his illness, the severity of the symptoms brought the patient to the hospital.

On entrance there was orthopnoea and severe paroxysms of coughing. There was fever. The heart was enlarged and showed obvious evidence of aortic stenosis and mitral insufficiency with failure of the right side—enlarged, pulsating liver, œdema of the extremities. There was grave anæmia (R.b.c., 2,832,000; hb., 47%) and a high leucocytosis (13,000-22,240). There was moderate albuminuria, and on several occasions blood in the urinary sediment. Irregular remittent and intermittent fever continued, 99° to 102.6°; persistent cough. Petechiæ appeared in the conjunctivæ. Death followed a sudden paroxysm of coughing, eight weeks after the onset of his illness.

Cultures from the blood during life were repeatedly negative.

The symptoms were regarded as indicating an ulcerative endocarditis of the aortic valve. The history suggesting pulmonary embolism (bloody expectoration) pointed to the possibility of involvement of the valves of the right side or thrombi probably in the right auricular appendage. The slow course of the case, eight weeks' duration, was suggestive of an infection with attenuated streptococci or gonococci, and the negative results of cultures supported the possibility of its being gonorrhœal.

At necropsy, not obtained, unfortunately, until five days after death, there was found old chronic, fibrous aortic and mitral stenosis without acute changes. Somewhat above the aortic valves, however, as shown in the photograph, there was an acute ulceration of the aorta leading to an aneurysmal sac from which there were accessory dilatations. Around the edges of the sac and at its base were vegetations. The aortic wall had been torn through, and from the sac there exuded a thick gray fluid showing Gram-negative diplococci and a few Gram-positive cocci. Elsewhere the wall of the aorta was normal. Owing to the condition of the specimens, no cultures were made.

The overwhelming prevalence in the smears of organisms presenting the morphological and tinctorial characteristics of gonococci, when taken in connection with the negative blood cultures *intra vitam*, led us to regard the process as gonorrhœal. There were no other evidences of acute gonorrhœa.

CASE III. Vegetative and ulcerative endocarditis of aortic valves—Cultures from circulating blood, negative—Gonococci obtained in pure culture from the valvular lesions at necropsy.

M.M., (Med., 45977; Path., 6681), a healthy married woman of 21 was seized ten weeks before death, with arthritis in knees.



CASE II. A. S. Gonococcal endoarteritis with the formation
of an aneurysm.



ankles and hip, associated with irregular remittent and intermittent fever. The arthritis which was apparently at no time very acute, disappeared in 10 days, but the fever continued. Signs of aortic insufficiency set in with very collapsing pulse but without marked cardiac enlargement. There was pronounced dyspnoea. The patient became anæmic, was dull and confused mentally, and was brought to the hospital three weeks after the onset of her illness. In the hospital there was irregular intermittent and remittent fever (98° - 103.5°); progressively increasing anæmia (R.b.c., 4,236,000 to 2,464,000; hb., 58% to 28%), and a marked leucocytosis (13,520 to 39,080). There were no petechiæ and no obvious embolic phenomena. The pulse increased steadily in rate. Striking and rapid changes occurred in the heart sounds and in the character of the pulse which was at first very collapsing, later much less so, while the intensity of the diastolic murmur diminished and the first heart sound became strangely accentuated at the base.

Persistent delirium; fever; tachycardia. Progressive failure. Evidence of chronic passive congestion (enlarged liver). Blood cultures, repeatedly negative. Death ten weeks after onset.

At first it was thought that the case might be an instance of acute rheumatic endocarditis, but the remarkably sudden onset of an aortic insufficiency of so high a degree and the relatively small heart—for, in the young, hypertrophy soon follows an aortic insufficiency of any extent, suggested an ulcerative endocarditis. This was borne out by the subsequent course of the case—the remittent, high fever, the progressive anæmia, the high leucocytosis and especially the sudden changes in the character of the heart sounds.

As we put together the facts, we could not help being impressed by the possibility that this might have been an instance of gonorrhœal endocarditis. In several similar cases in which cultures *intra vitam* were persistently negative, this has proved to be the case.

Necropsy showed an extensive vegetative and ulcerative endocarditis of the aortic valves, with large, white, crumbling vegetations, perforations of the valves, and an abscess extending from the valvular lesions into the muscle of the left ventricle. There was no evidence of pre-existing valvular disease, one curtain remaining delicate and unaffected. There were fresh splenic infarcts and small hæmorrhages into the pulp. The kidneys were large. There were old adhesions between the omentum and tubes and round ligaments, and thrombi in the pampiniform plexus. Gram-negative intracellular diplococci were demonstrated in smears, and pure cultures of gonococci were obtained from the focal lesions on the valves.

Since the opening of the Johns Hopkins Hospital, nearly thirty-three years ago, there have come to necropsy 327 instances of acute endocarditis. In 133 cases the nature of the process was determined bacteriologically,* either before death by blood cultures, or at necropsy. To these may be added 16 fatal cases in which necropsy was not obtained, although the nature of the process was determined by cultures from the blood *intra vitam*, as well as 27 cases with positive blood cultures in which the patients were removed from the hospital unimproved, making 176 altogether.

Twenty, or 11.3% of these instances, were due to gonococcus.

* There were others in which a bacteriological study was made, but the records and reports were destroyed in a recent fire in the Pathological Laboratory.

In 4 of these 20 cases, there were terminal infections; in 2 with streptococcus; in one with streptococcus and staphylococcus, and in one with pneumococcus. In all, gonococcus was evidently the primary cause.

Of these twenty cases, gonococci were cultivated *intra vitam* (10) or at necropsy in 14 instances.

Gonococci were demonstrated bacterioscopically at necropsy following negative cultures *intra vitam* or *post mortem* in 6.

There were two further cases which, clinically and anatomically, seemed to be gonorrhœal, with negative cultures *intra vitam* in which, owing to a misunderstanding, no bacteriological examination was made at death. These we have included in some of our tables.

In addition, there were several other cases in which the endocarditis may well have been gonorrhœal, although definite proof was wanting. One was a characteristic instance of ulcerative endocarditis appearing in the course of an acute gonorrhœa with negative blood cultures during life. No necropsy was allowed. These cases have not been used in the tables, although in the last instance the course of the illness, the negative blood cultures, and the sequence on gonorrhœa, render the diagnosis of gonorrhœal endocarditis highly probable. The summary of this case may be found in the appendix.

This is a rather large material for one clinic, dependent, probably, on the circumstance that we have been on the lookout for the condition.

Age: Of these patients the age varied from 9 to 42.

Sex: There were 16 males; 6 females.

Colour: Eleven were white, eleven were negroes—a large proportion of negroes, larger than was observed in other forms of endocarditis excepting pneumococcus infection.

Time of onset in relation to the gonorrhœa: But little evidence could be obtained as to the relation of the onset of the symptoms to the stage of the initial local infection. Among our twenty-two cases the cardiac complications appeared almost with the onset of the gonorrhœa in one instance. In four they appeared about two weeks after the onset; in two more within a month; in the others (fifteen) the relation to the time of onset of the gonorrhœa was uncertain. In several instances all traces of the urethritis had disappeared.

Relation of the onset of cardiac involvement to the other complications of gonorrhœa: In like manner little information was thrown on the relation of cardiac involvement to the occurrence of other complications of gonorrhœa.

Of our twenty-two cases but seven or 31.1% had had arthritis in the course of the illness. This is in sharp contrast to the figures in cases reported by other authors. Among 54 instances of proven gonorrhœal endocarditis collected from the literature in which there were notes as to the presence or absence of involvement of the joints, arthritis was noted in 37, or 68.5%. It is not impossible that this variance depends on the circumstance that where

routine bacteriological examinations are not made, the disease, in the absence of obvious urethral discharge or arthritis, is often overlooked.

Symptoms: The symptoms were uniformly those of a grave septicæmia. The onset was sometimes gradual, beginning with headache, lassitude, pains in the loins, or more sudden and initiated by a sharp chill. There was continued or irregularly remittent or intermittent fever, often associated with violent chills and sweating. *Chills* were present in the majority (63.6%) of our cases.

Blood: There was, as a rule, a rapidly developing anæmia. The red corpuscles ranged from 5,467,000 to 1,248,000 per c.mm.; the hb. from 92 to 17%. The longer the duration of the case, the more marked, as a rule, was the anæmia.

In all cases there was a well-marked leucocytosis. In but two instances were figures below 13,000 recorded and in each instance there was a leucocytosis later.

The leucocytes were over 15,000 in 19 of 22 cases.

The leucocytes were over 20,000 in 14 of 22 cases.

The leucocytes were over 30,000 in 7 of 22 cases.

The leucocytes were over 40,000 in 4 of 22 cases.

In one instance with a terminal pneumococcus pneumonia and septicæmia the leucocytes rose to 78,000 and 100,000.

Embolic phenomena were common, occurring in two-thirds of the cases—in skin, kidney, spleen, lungs, brain, heart muscle, skeletal muscles.

Symptoms of these embolic phenomena were not uncommon during life. *Pulmonary infarction*, for instance, gave rise to the initial symptoms in the first of the three cases described at the beginning of this communication. *Renal infarction* may be suspected by the transient presence of blood in the urine. Blood corpuscles were found in the urine of 10 of our cases. But occasional blood corpuscles are not uncommon in the nephritis so frequently present. Pain and tenderness in the left hypochondrium may reveal *splenic infarction*. In two of our cases there was a partial *hemiplegia*.

Cutaneous hemorrhages, usually in the form of petechiæ on limbs or trunk or neck or mucous membranes of the mouth or conjunctivæ, were frequent. These were recorded in 6 of our 11 white patients—in but one of the negroes. Had the conjunctivæ and mucous membranes been examined with greater regularity 'tis probable that these figures would have been different.

An *acute or subacute nephritis* was present in the majority of our cases. This was manifested clinically by the presence of a considerable albuminuria with casts, leucocytes, epithelial cells and often blood in the urinary sediment. In 4 cases of long duration there was anasarca and hydrops. In general, the renal manifestations were more striking in the cases of longer duration.

The duration of our gonorrhœal endocarditis varied from 3-4 weeks to 8 months, averaging 9.7 weeks. If one

subtract four cases in which there was a terminal mixed infection, the average duration was 10.5 weeks. In the majority of instances the course of the disease was from 4 to 9 weeks. The shortest case, the duration of which was not over 4 weeks, was that of a little girl with gonorrhœal peritonitis in which death was not the result of the endocarditis. In many of our cases, the picture was that of subacute vegetative endocarditis similar to that seen in infection with streptococcus viridans.

A comparison of these figures with those of endocarditis of other origin is not uninteresting.

TABLE I

Duration of fatal cases of acute bacterial endocarditis.

	Pneumococcus 4 d.-6 m. Av. 4.3 w.	St. aureus 3 d.-6 m. Av. 4.5 w.	St. albus 3-11 w. Av. 6.8 w.	Gonococcus 3 w.-8 m. Av. 10.5 w.	Strep. all strains few d.-9 m Av. 12.2 w.	Strep. vir. 3 w.-1 y. Av. 23.1 w.	Influenza 11-26 w. Av. over 6 m.
Under 13 weeks	21/22	20/24	4/4	18/22	37/60	4/22	1/3
" 9 "	20/22	20/24	2/4	16/22	34/60	3/22	—
" 6 "	17/22	20/24	2/4	8/22	31/60	3/22	—
" 3 "	11/22	14/24	0/4	1/22	20/60	0/22	—
" 1 week	4/22	5/24	—	—	10/60	—	—

Pneumococcus, in our experience, produces an acute infection, one half of our cases pursuing their course in less than 3 weeks. In but one of 22 cases was the duration above nine and a half weeks.

Staphylococcus aureus is not infrequently a terminal invader and in over half of our cases the process was rapidly fatal with a duration of less than three weeks. The duration was under 6 weeks in 83% of our cases. But while *staphylococcus aureus* endocarditis appears generally to be more acute and virulent than gonococcal, yet in 4 of our 24 cases the course was subacute, lasting from 13 weeks to 6 months.

There were four instances of pure *staphylococcus albus* endocarditis, the duration of which varied from 3 to 11 weeks, the average course being somewhat more chronic than in aureus infections.

In our group of streptococcus infections, because of the circumstance that our records go back through so long a series of years, during most of which no differentiation of type was made, all strains of the organism are included. It is not therefore surprising to find a great variation in the duration of the disease. Thus 20 of 60 cases were peracute, of under 3 weeks' duration, 11 of these running their course in a week or less. On the other hand 23 of the cases or over one-third lasted 13 weeks or over. Of these, all were probably instances of infection with streptococcus viridans.

In 22 cases in which *S. viridans* was identified bacteriologically, the duration ranged from 3 weeks to a year, averaging 23.1 weeks. In only 3 instances in which streptococcus viridans was isolated was the duration under 13 weeks, and in one of these instances it was a

terminal infection in pernicious anaemia. It would appear that hæmolytic streptococci are associated generally with rapidly fatal infections.

There were three fatal cases of endocarditis due to organisms of the *B. influenzae* group. These were all chronic, lasting from 11 to 53 weeks.

Gonococcus seems to give rise to an endocarditis of a malignancy between that depending on staphylococcus and pneumococcus on the one hand and the slower viridans and influenzal infections on the other. The rapidity of the course and malignancy of bacterial endocarditis rises, in our experience, in the following sequence:

1. *B. influenzae*
2. *Streptococcus viridans*
3. *Gonococcus*
4. *Staphylococcus albus*
5. *Staphylococcus aureus*
6. *Pneumococcus*
7. *Streptococcus hæmolyticus*

Anatomical Lesions: The anatomical lesions were those of a vegetative and ulcerative endocarditis, sometimes spreading to the walls of the ventricles or upwards into the sinuses of Valsalva, associated often with extensive destruction of valves and aneurysm formation, sometimes infiltrating the adjacent muscle as suppurative myocarditis. In general, the vegetations formed large, rather brittle, yellowish gray masses.

The only instance in which the vegetations were not large was that of a girl of 9 who died of a general gonococcal infection with peritonitis. Here the lesions were on the wall of the aorta above the valves and were of short duration—not over four weeks—probably somewhat less. In one other instance—that of A. S., described at the beginning of this communication—the lesions were those of a vegetative and ulcerative aortitis.

The abundance and size of the vegetations were commonly in direct relation to the duration of the case.

Mural endocarditis: The vegetations extended onto the wall of the heart in 8 of 20 cases, involving the left ventricle, papillary muscles and chordæ tendineæ in 5 cases, the right side in 4, the left auricle in one.

Vegetative and ulcerative aortitis: Two of the twenty cases coming to necropsy showed a vegetative and ulcerative aortitis alone. In two other cases, in addition to the valvular lesions, there were vegetations on the aortic wall.

Acute pericarditis occurred in four of the cases.

In one case there was a peri-aortic suppuration in the loose connective tissue just above the valves. There was *pleurisy* in two cases, in one of which gonococcus was cultivated from the fibrino-purulent fluid.

Our figures, as might be expected, show that the left side of the heart is affected more often than the right, but the percentage of right-sided involvement is remarkably high, considerably higher than in those cases of gon-

TABLE II
Anatomical distribution of the lesions.

	Gonococcus			St. aureus 24 cases	St. albus 4 cases	Pneumo- coccus 25 cases	Strepto- coccus 52 cases	
	J. H. H. series 20 cases	Literature 60 cases	Combined 80 cases					
Left side	A	6	30	6	2	9	9	
	M	2	15	17	1	4	17	
	AM	2	8	10	4	0	16	
	Aorta, mural	2	0	2	0	0	0	
		12-60%	53-88.3%	65-81.2%	17-70.8%	3-75%	19-76%	42-80.7%
Right side	P	4	1	5	1	0	1	
	T	1	0	1	5	1	1	
	PT	0	1	1	0	0	0	
		5-25%	2-3.3%	7-8.7%	6-25%	1-25%	2-8%	3-5.7%
Both sides	AP	0	1	1	0	0	0	
	AT	2	1	3	1	0	1	
	MT	0	0	0	0	0	0	
	APM	0	1	1	0	0	0	
	APT	1	0	1	0	0	1	
	AMT	0	1	1	0	0	3	
	AMPT	0	1	1	0	0	0	
		3-15%	5-8.3%	8-10%	1-4.1%	0	4-16%	7-13.4%

orrhæal endocarditis that we have collected from other sources. This high proportion of affection of the right side was also found in staphylococcus endocarditis, and is in rather sharp contrast to that which is shown by the figures for pneumococcus and streptococcus. In general, however, it is in accord with the well-recognized relative frequency of acute ulcerative lesions of the right side of the heart.

TABLE III
Distribution of the affection among the individual valves.

	Gonococcus		St. aureus 24 cases	St. albus 4 cases	Pneumo. 25 cases	Strep. 52 cases	S. viridans 13 cases
	J. H. H. series 20 cases	Total cases collected 80 cases					
A	13-65%*	56-70%	12-48%	2-50%	19-76%	32-61.5%	8-61.5%
M	4-20%	30-37.5%	11-44%	1-25%	13-52%	39-75%	11-84.6%
P	5-25%	10-12.5%	1-4%	1-4%	1-4%	4-7.6%	1-7.6%
T	4-20%	8-10%	6-24%	1-25%	6-24%	9-17.3%	

* Including two instances of mural aortitis.

Of the individual valves the aortic was affected with greatest frequency; the pulmonary next, in a quarter of our cases. This is somewhat striking and again in contrast to the usually accepted figures which show a somewhat larger percentage of aortic involvement—70% in the combined 80 cases of reasonably proven gonococcal endocarditis that we have collected.

One striking point, however, that stands out in these figures is the greater frequency of the involvement of the mitral valve in streptococcal infections than in gonococcal, staphylococcal and pneumococcal endocarditis.

The probable explanation of this divergence is found when we consider:

(1) The relative frequency of pre-existing valvular disease so far as could be determined by the histories and the anatomical changes.

(2) The relative frequency with which previously unaffected valves were involved.

TABLE IV
Evidence of pre-existing valvular disease.

Gonococcus	Staph. aureus	Staph. albus	Pneumococcus	Streptococcus
4/20 or 20%	10/24 or 41.6%	1/4 or 25%	9/25 or 36%	35/52 or 67.3%

There was evidence of pre-existing valvular disease in only 20% of our gonorrhœal cases; in 41.6 of the staphylococcus aureus cases; in 25% of the four albus cases; in 36% of the pneumococcus infections as against 67.3% for our streptococcus cases. This is a striking contrast. 'Tis clear that acute streptococcus endocarditis was commoner in patients with chronic valvular disease, whereas the contrary was true in infections with the other organisms.

If, now, we consider, among the valves themselves, the relative frequency in which the affection was found on previously undiseased valves, it appears that the valves involved were, so far as could be determined, previously unaffected in the following percentages of the several commoner types of infection.

TABLE V
Per cent in which the acute process involved previously unaffected valves.

Gonococcus*	Staph. aureus	Staph. albus	Pneumococcus	Streptococcus
91.2%	65.5%	75%	65.7%	39.2%

* Not including the mural aortitis.

In other words, in more than half of our cases, 61%, Streptococcus attacked previously diseased valves—in sharp contrast to the behaviour of the other chief causal agents of acute endocarditis, notably of Gonococcus. *But the prevalence of mitral involvement in chronic endocarditis is well known.* If, as seems probable, Streptococcus attacks, generally, previously diseased valves, its predilection for the mitral valve may well depend largely on the circumstance that chronic mitral disease is very common.

Further light on this question might be obtained by a consideration of the relative percentage in which the individual valves are involved among those primarily affected.

TABLE VI
Relative percentage of involvement among those valves primarily affected.

	Gonococcus	Staph. aureus	Staph. albus	Pneumococcus	Streptococcus
A	10* 45.4%	4-21%	1-33.3%	13-52%	12-37.5%
M	4 18.1%	8-42.1%	1-33.3%	9-36%	14-43.7%
P	5 22.7%	1-5.2%		0-0	1-3.1%
T	3 13.6%	6-31.6%	1-33.3%	3-12%	5-15.6%

* Not including the two mural aortides.

The figures would appear to show a tendency toward aortic involvement in gonococcal and pneumococcal infection, toward involvement of the mitral in infections with streptococcus and staph. aureus. But the number of cases on which the table is based is so small as to render it practically worthless.

In summary, then, the cardiac complications of gonorrhœa, as we have seen them, appear usually in the form of an acute or subacute vegetative and ulcerative endocarditis which comes on at varying periods in the course of the infection, often in the absence of other apparent complications. The onset may be sudden or more gradual, but is generally rather acute. There are usually chills and high remittent or intermittent fever, rapidly developing anæmia and considerable leucocytosis.

The constitutional symptoms, those common to all grave general infections, are usually early and profound. There is often a petechial eruption. Embolic phenomena of various sorts are common. There is albumin, casts, often blood in the urine, and, in the longer cases, anasarca and hydrops give evidence of a grave nephritis.

The duration is generally from 5 to 9 weeks, but longer subacute cases may occur.

The aortic valve is that most commonly involved but, as in all acute ulcerative endocarditides, the right side appears to be affected more often than in the chronic so-called rheumatic valvular disease. In our experience involvement of the pulmonary orifice has been rather common. Gonococcus usually involves healthy, previously unaffected valves. Mural endocarditis is common. Suppurative myocarditis, as described in 1893 by Councilman, may occur. Pericarditis is not infrequent.

Gonorrhœal endocarditis is generally a malignant process pursuing a progressive and fatal course. In its virulence it occupies a position between the slower and more subacute viridans and influenza infections and the more virulent albus, aureus, pneumococcus or s. hæmolyticus endocarditis.

But is gonorrhœal endocarditis always fatal? May recovery occur?

If so, what is its frequency and what is the prognosis?

Gonorrhœal septicæmia with every evidence of endocarditis is not necessarily fatal. Characteristic instances of acute endo- or peri-carditis have been recorded in which gonococci were cultivated from the blood during life with subsequent recovery. Good examples are the cases reported by Silvestrini, Withington, Dieulafoy, and Marfan and Debré.

Silvestrini records the case of a man, 29 years of age, who acquired gonorrhœa a month before entry into the hospital. He was treated with injections of wine—an unworthy abuse of a noble fluid. Eleven days after apparent recovery, anorexia, malaise, chills and sweats set in. On the third day there were pains in the joints of the

spine with swelling and redness of the accessible joints. The patient's appearance was typhoidal. In the sub-clavicular fossæ there appeared erythematous patches with central hæmorrhages. The cardiac impulse was in the fifth space in the mammillary line. There was an intense systolic murmur in the mitral area, and a doubled second sound. A pericardial friction rub was audible. The spleen was enlarged. There was a well-marked leucocytosis. Gonococci were cultivated from the blood, joints and urethral discharge. The pericardial friction disappeared and the patient gradually recovered.

The heart diminished in size but a harsh systolic murmur at the apex and a reduplicated second sound persisted. The patient left the hospital with ankylosis of several joints, and returned nine months after the onset of his trouble, still showing evidence of chronic mitral disease with systolic and presystolic murmurs.

Withington had the opportunity of observing a man of 26 who acquired gonorrhœa and, two weeks later, measles. Soon after convalescence from measles, præcordial pain set in, associated with chills, irregular fever and orthopnoea. There was cardiac enlargement, gallop rhythm and evidence of solidification at the bases of the lungs. There was a left femoral thrombo-phlebitis. A pericardial friction rub appeared and a short systolic murmur was heard in the fourth intercostal space to the left of the sternum. Cultures from the blood showed gonococcus.

Gradually the patient recovered, the temperature reaching normal about three weeks after the onset of the symptoms pointing to cardiac involvement. The heart returned almost to its normal size but a systolic murmur persisted in the second left space.

Dieulafoy reports the case of a man of 23 who was admitted to the hospital with headache, diarrhœa, high remittent fever and sweating. Two days later a mitral systolic murmur appeared, soon becoming loud and rough. There was an urethral discharge in which gonococci were present. Cultures from the blood, *intra vitam*, showed gonococci. The patient was very ill. There was bronchopneumonia. After treatment by rest and gonococcal vaccine, the patient gradually recovered and left the hospital well. The evidence of a mitral lesion remained.

Marfan and Debré report the case of a little girl, aged 10½, who, about a week before entry into the hospital on 24/VI, began to suffer from pain in the abdomen, fever and exhaustion. There was obvious pelvic peritonitis. The heart was somewhat enlarged with a slight systolic thrill and an intense, rough, systolic murmur transmitted outwards into the axilla. Five days after entry there was a pericardial friction murmur. High, irregular fever. Cultures from the circulating blood showed characteristic gonococci.

Treatment with gonococcic vaccine and anti-meningococcic serum. Gradual recovery after about 3 weeks, with

persistent signs of mitral insufficiency and adherent pericardium. Ten months after the beginning of her illness the child still showed signs of adherent pericardium and mitral insufficiency.

Gourvich, in 1897, in an excellent study of the literature which has passed almost unnoticed, brought together a considerable number of instances in which transitory cardiac manifestations, interpreted as endocarditis, had been observed in the course of gonorrhœa. Many of these are suggestive but most consist of the description of systolic murmurs at the apex of the heart appearing during the height of the process and disappearing with recovery. Similar cases have been reported by other observers since the article of Gourvich.

The recognition of mild, early endocarditis is often very difficult, indeed impossible. Systolic murmurs at the apex and pulmonary areas are commonly heard in the course of any acute infection, and, in themselves, offer little basis for the diagnosis of endocarditis. In many instances of rheumatic fever 'tis only through long continued observation of the patient that a positive diagnosis of endocarditis is reached, and then only on the strength of the later mechanical effects of scarring and retraction of the valves in the production of permanent stenoses and insufficiencies.

The very frequency of gonorrhœa contributes to the difficulty of ascertaining its relation to endocarditis. Few genito-urinary surgeons, probably, examine the hearts of their patients as a routine procedure, and without knowledge of the condition of the heart at the time of onset of the gonorrhœa, it may be difficult or impossible to reach a conclusion as to the relation of the malady to a chronic valvular disease which is recognized later.

Our impression, and it must be acknowledged that it is but an *impression*, is, that the analogy between the cardiac complications of gonorrhœa and those occurring in pneumococcus and staphylococcus infections is close; that while mild infections with a favorable course may occur, gonorrhœal endocarditis is generally a serious affair, in no way comparable to the acute, verrucous, so-called rheumatic endocarditis of uncertain origin, which affects so commonly the mitral valves, and tends toward recovery with scarring, deformity, and the production of chronic lesions which are notoriously a seat of predilection for later streptococcal invasion with the development of acute and subacute vegetative and ulcerative lesions.

How often endocarditis occurs in gonorrhœa we have no clear idea. This, however, we do know. Gonorrhœal endocarditis is not a very infrequent malady, forming upwards of 11% of our fatal acute endocarditides, the bacterial nature of which has been determined at necropsy.

SUMMARY AND CONCLUSIONS

The cardiac complications of gonorrhœa occurring in thirty-three years at the Johns Hopkins Hospital have been reviewed. Twelve hitherto unreported cases have

been added to our series * and considered in connection with sixty cases collected from the literature.

In 176 instances of acute endocarditis of determined origin 20, 11.3%, were gonococcal.

Gonococci were cultivated *intra vitam* (10) or *post mortem* in 14 instances; they were demonstrated bacterioscopically following negative cultures *intra vitam* or *post mortem* in 6 instances.

There were two additional cases, in all probability gonorrhæal, with negative cultures during life in which no bacteriological examination was made at necropsy, and several other instances of more or less characteristic endocarditis occurring in the course of acute gonorrhæa with negative blood cultures but without necropsy; these latter cases were not considered in our tables.

But little evidence could be obtained as to the relation of the time of onset of the cardiac involvement to the appearance of the disease or other complications. Arthritis occurred in but 41.1% of our cases but in 68.5% of 54 cases collected from other sources.

The cardiac complications of gonorrhæa, as we have seen them, appear usually in the form of an acute or subacute vegetative and ulcerative endocarditis which comes on at varying periods in the course of the infection, often in the absence of other apparent complications. The onset may be sudden or more gradual, but is generally rather acute. There are usually chills and high remittent or intermittent fever, rapidly developing anemia and considerable leucocytosis. The constitutional symptoms, those common to all grave general infections, are usually early and profound. There is often a petechial eruption. Embolic phenomena are common. There is albumin, casts, often blood in the urine, and in longer cases, anasarca and hydrops give evidence of a grave nephritis.

The duration is usually from 4-9 weeks but longer subacute cases may occur.

The aortic valves are those most commonly involved but, as in all acute endocarditides, the right side appears to be affected more often than in the chronic, so-called rheumatic valvular disease. In our experience, involvement of the pulmonary orifice has been rather common. *Gonococcus* usually implicates healthy, previously undiseased valves. Mural endocarditis is frequent.

The cardiac involvement is not restricted to the endocardium. Endoarteritis, pericarditis and suppurative myocarditis are not unusual.

Gonorrhæal endocarditis is in general, a malignant process pursuing a progressive and fatal course. In its virulence it occupies a position between the slower and more subacute *S. viridans* and *B. influenza* infections and the more virulent *Staph. albus*, *Staph. aureus*, *Pneumococcus* or *S. hæmolyticus* endocarditides.

Milder instances of gonorrhæal endocarditis or pericarditis with recovery may occur. How frequent these are is not clear. It is our impression that they are relatively rare.

Gonorrhæal cardiac infections as a whole are by no means very unusual.

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* See appendix.

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APPENDIX

Ten of our twenty-two cases of gonorrhoeal endocarditis have already been reported by Thayer and Blumer, Thayer and Lazear, Harris and Dabney, Harris and Johnston and Thayer. In addition to the three cases reported in the body of the article there remain nine which are here given in summary. An additional tenth case with negative cultures during life which did not come to necropsy is also summarized because of its characteristic course.

CASE IV. *Pregnancy—Ulcerative endocarditis of mitral (?) valve. Pericarditis. Gonococci obtained in pure culture from circulating blood. Obstinate vomiting. Abortion. Death. Necropsy refused.*

A. B., (Med., 18490; 18505.) A healthy woman of 39 who had four children, began, on the first week of May 1905, to suffer with stiffness of the legs and arms and, 4 days later, with pain,

tenderness and swelling of the dorsum of the right hand. Several days later, chills, fever and severe headache set in, recurring daily and associated with vomiting and soreness in the epigastrium. She was about 6 months pregnant. She entered the hospital something over three weeks after the onset of the illness. There was anæmia (R.b.c., 3,700,000-2,907,000; hb., 65%-42%). Moderate leucocytosis (10,800-16,800). The urine showed a trace of albumin and many leucocytes and hyaline and granular casts. Heart, enlarged; loud systolic murmur at the apex, transmitted into the axilla and the back; second sound accentuated in the pulmonary area.

The patient remained in the hospital for the greater part of the next 5 weeks. There was high intermittent fever with chills. The leucocytes rose at one time to 31,000. The anæmia was progressive. Petchie appeared on the trunk. Jaundice set in. Delirium. Progressive enlargement of the liver which, on the 15th of June, was 8 cm. below the costal margin in the mammillary line. The patient aborted on the 17th of June without change in her symptoms. Obstinate vomiting. Friction rub over the præcordium. On the 18th of June, about 6 weeks after the onset of symptoms, she died.

Vaginal smears were negative for gonococci. After two negative blood cultures, on 12 June, 12 cc. of blood which clotted in the syringe was expelled into a flask of bouillon and a flask of milk. In both flasks there appeared, in the clot, colonies which proved to be characteristic gonococci; these were transferred successfully to hydrocele fluid agar and human blood serum. Necropsy refused.

CASE V. *Gonorrhœa. Arthritis. Ulcerative aortic endocarditis. Gonococci obtained in pure culture from circulating blood. Death—no necropsy.*

D. W. C., Jr., (Med., 18987) a horseshoer, aged 26. Second attack of gonorrhœa, 9 weeks before entry. Three weeks later, pain, swelling and redness in right knee-joint, and later in the muscles of the legs and right big toe-joint. Headache. Recurring chills and fever. Shortness of breath. Præcordial pains. Cough.

Entered the hospital on 13/VII/06, six weeks after the onset of the arthritis. At this time there was remittent fever (99°-103.8°) and leucocytosis (16,200-32,800). The heart was somewhat enlarged. Loud, roaring systolic murmur at the apex, transmitted outwards, with a diastolic murmur along the left border of the sternum; to and fro murmur in aortic and pulmonary areas; collapsing pulse. There was no urethral discharge. The urine showed a trace of albumin, occasional granular casts and a few red blood corpuscles in the sediment. The spleen was not palpable. There were no petchiæ.

Gonococci were isolated in pure culture from the circulating blood. Anti-gonococcus serum was administered without result.

The patient became very dyspnoic; the anæmia increased. On 24 July, 7-8 weeks after the onset of his symptoms, the patient lapsed into unconsciousness and died. Necropsy refused.

CASE VI. *Gonorrhœa. Vegetative and ulcerative aortic endocarditis. Cultures from blood during life, negative. Gonococci bacterioscopically in valvular vegetations.*

J. C., (Med., 20061; Path., 2765.) A coloured labourer with an history unimportant excepting in that, three weeks before, he had had a swollen ankle. Urethral discharge for two weeks. At about the time of onset of his urethritis was seized with a chill, præcordial pain, general weakness, headache and fever. On admission on 20/VIII/06, he was a healthy looking man with high fever, rapid pulse and a heart, slightly enlarged with a well marked systolic thrill at the apex, a rather flapping first sound and a loud, rough systolic murmur followed by a faint diastolic rumble, more intense in the aortic area and along the left sternal margin. Pulse, collapsing. Liver three fingers' breadths below the costal margin. Spleen, not felt. The leucocytes ranged

from 28,700 to 40,100 per cu. mm. Anæmia (R.b.c., 3,404,000; hb., 50%). The urine showed a trace of albumin; hyaline and granular casts. The temperature was remittent and sharply intermittent, ranging from normal to 105.6°. The patient failed steadily and died on 10/IX, about 5 weeks after the onset of his symptoms. Cultures from the circulating blood (blood agar and litmus milk) were negative on four occasions.

At necropsy the pericardial cavity contained about three times the normal amount of fluid. The endocardium of the left ventricle close to the aortic valve showed a few scattered yellowish-white vegetations. The three cusps of the aortic valve were greatly distorted, thickened and covered with an irregular mass of fragile yellowish-white vegetations. A few scattered vegetations were also seen on the intima of the aorta posteriorly and scattered over the intima of the ascending arch. Anæmic infarcts of spleen and kidneys. Cloudy swelling of all the viscera.

Smears from vegetations on the aortic valve showed Gram-negative diplococci, morphologically and tinctorially gonococci. Cultures from the pericardial fluid and heart's blood, negative.

Diagnosis: acute gonorrhæal endocarditis.

CASE VII. *Gonorrhæal urethritis—cystitis—endometritis—salpingitis. Vegetative and ulcerative aortic and mitral endocarditis. Pericarditis. Vegetative aortitis. Mural endocarditis. Pure cultures of gonococci from circulating blood and pericardium during life.*

C. C., (Med., 21977; Path., 2981) a house-maid, aged 21, gave birth on 25/XI/07, to a full-term child. Two days later, despite the presence of a fever of 102°, she rose from bed and insisted on going to work. Fever continued. Extensive vaginal and urethral discharge. Finally, she was admitted to the gynecological service where she developed a pericarditis. On entrance to the medical side on 19 Dec., she was very anæmic (R.b.c., 2,320,000; leucocytes, 29,560; hb., 41%). There was an intense pericardial friction and a loud to and fro murmur in the aortic area. The friction murmur almost obscured the other cardiac sounds. There was a chronic arthritis of the left hip-joint with a discharging sinus. The temperature was continuous, 99.2°-102.4°; the pulse rapid. The urine showed considerable albumin, many leucocytes and occasional red corpuscles.

Cultures from the blood on the day after entry showed, on blood agar plates, many small colonies which proved to be characteristic gonococci.

The patient failed rapidly. On 24/XII a cloudy fluid with Gram-negative diplococci was obtained from the pericardium. On Christmas day, about 8 weeks after the onset of her symptoms, she died.

The necropsy showed a sub-acute gonorrhæal urethritis, cystitis, endometritis, and salpingitis. There was a subacute fibrinous pericarditis with effusion, and bilateral pleurisy. Along the line of closure of the mitral valve there were 3 pin-point, grayish elevations. The posterior aortic cusp was covered by a large gray mass about the size of a terminal phalanx of one's finger, which was very friable. On the left cusp there were several masses of similar appearance and consistency measuring several millimeters in diameter; these were also seen at the base of the aorta and on the ventricular endocardium near the aortic orifice. The kidneys were enlarged, cortex thickened and pale. The liver was rather large, and showed evidences of central necroses and degeneration.

Smears from the aortic vegetations, pericardial and pleural fluids, tubes and endometrium showed gonococci which were also found but in small numbers in an area of bronchopneumonia.

CASE VIII. *Vegetative and ulcerative endocarditis of mitral and aortic valves—Arthritis. Multiple embolisms. Gonococci obtained in pure culture on two occasions from the circulating*

blood and demonstrated morphologically and tinctorially in the valvular thrombi.

H. B., (Med., 22529; Path., 3050.) a negro aged 25, denying venereal disease, had been seized 7 weeks before entry with a chill, and on the following day, with pains in his knees and legs. This had been followed by fever and a slight cough. From the onset he had been obliged to stay in bed.

On admission on 24/IV/08, he was a well developed coloured man, not especially anæmic (R.b.c., 5,300,000; hb., 72%); there was a marked leucocytosis (19,300) and high fever with rapid pulse. The heart was a little enlarged. There was a palpable systolic thrill. At the apex the first sound was replaced by a high-pitched, long, blowing murmur; second pulmonic, accentuated. The temperature ranged from subnormal to 105°; sharp intermittent paroxysms. Urine: sp. gr., 1015-1020; trace of albumin; in the sed., hyaline and epithelial casts and occasional red blood corpuscles. The leucocytosis increased, reaching at one time, 33,900. On the first of May there was a severe nose-bleed. On 2/V small conjunctival ecchymoses appeared. On the same day there was a sudden loss of power on the left side with increased reflexes. A diastolic murmur developed along the left border of the sternum. The patient failed rapidly, the urine became scanty, the pulse, more rapid, and on 2/V, about 8 weeks after the onset of his illness, he died.

Gonococci were twice obtained in pure culture from the circulating blood. At necropsy there were numerous subepicardial hæmorrhages. The mitral orifice was almost plugged by a large thrombus attached to the right half of the aortic cusp, measuring 2.5x1.5 cm.; there was another mass of about the same size on the left half of the ventricular cusp of the valve. Many of the chordæ tendineæ were covered with small, translucent, wart-like growths as were the papillary muscles supplying these chordæ. About 1 cm. above the point of attachment of the mitral valve on the endocardium of the auricle, there were several wart-like vegetations. On the posterior aortic cusp was a small thrombus mass attached by a pedicle. There were multiple emboli in the brain. Anæmic infarcts in the kidney, conjunctiva, heart and muscles. Kidneys, large, showing marked parenchymatous degeneration. Morphologically and tinctorially gonococci were demonstrated in the thrombi on the valves.

CASE IX. *Vegetative and ulcerative endocarditis of aortic and tricuspid valves. Gonococci obtained in pure culture from the circulating blood and demonstrated microscopically in the valvular thrombi.*

W. W., (Med., 25152; Path., 3337.) a coloured janitor, began on 31/XII/09 to suffer from fever, exhaustion, cough with considerable expectoration, sweating at night. At the end of a week he was so feeble that he went to bed. On entry, two weeks after the onset of his symptoms, there was high fever, rapid pulse, marked leucocytosis, ranging, during his illness, from 15,240 to 33,600; moderate anæmia (R.b.c., 4,684,000; hb., 75%), albuminuria and cylindruria.

Heart, slightly enlarged; the first sound at the apex, booming followed by a loud systolic murmur transmitted to the axilla; faint diastolic heard along the left sternal margin. Pulse, rapid and collapsing. Liver, enlarged, 2 cm. below the costal margin. Culture from the blood on entry negative.

The patient steadily failed. There was high remittent and intermittent fever and increasing rapidity of the pulse. On 19 Jan., cultures from the blood by Dr. Guthrie showed characteristic gonococci. Sudden death on 21/I about three weeks after onset of symptoms.

Necropsy: Just at the attachment of two cusps of the tricuspid valve, below the foramen ovale, was a wart-like vegetation, friable and firmly adherent to the wall. A few small vegetations on left coronary cusp of the aortic valve. The right coronary and pos-

terior cusps of the aortic valve showed large excrescences and ulcerations one of which had extended backward for some distance into the heart wall. There was an acute diffuse nephritis; splenic infarction; bilateral hydrothorax. Bacterioscopically, gonococci were found in the smears from the thrombi.

CASE X. *Gonorrhœal vaginitis, metritis, salpingitis, pelvic peritonitis. Hemiplegia. Vegetative aortitis. Blood cultures, negative. No bacteriological examination at necropsy.*

G. W. (Surgical, 27762; Path., 3556.) a coloured girl aged 9. Five days before entry, pain in the lower abdomen and about the umbilicus with cramp-like exacerbations; vomiting. Entered the hospital on 9/V/11, with severe abdominal pain, moderate distention, tenderness and muscle spasm. Temperature, 99.8°. Physical examination, otherwise negative. Leucocytes, 10,000-12,000. Purulent vaginitis with Gram-negative intracellular diplococci, morphologically characteristic of gonococci. Irregular remittent fever (99-102°), slowly rising.

22/V. The patient suddenly became stuporous, did not answer questions; tossing the head from side to side; pupils dilated; reflexes, exaggerated, ankle clonus; Babinski, positive. Lumbar puncture, clear fluid. Pulse, irregular. Loud systolic murmur in the pulmonary area.

24/V. Reflexes exaggerated on the left; rigidity more marked on the right. Abdominal reflexes, absent. Blood cultures and cultures from the spinal fluid negative. The pulse became steadily more rapid, the leucocytes increased to 13,000 and on June 1 the patient died. The specific gravity of the urine varied from 1005-1016; there was a trace of albumin; epithelial and pus cells in the sediment.

Necropsy: Acute urethritis, vaginitis, cystitis, pyelitis, endometritis, bilateral salpingitis; acute and chronic pelvic peritonitis. Heart valves and orifices showed no abnormalities. In the aorta "just above one of the sinuses of Valsalva is a small, round area, 4 mm. in diameter, on which the intima is lost and the wall covered by soft friable grayish material which extends into the lumen of the artery, 2-3 mm." Brain: "The basilar artery, posterior cerebrals and the right middle cerebral artery and some of its branches are occluded by red friable casts. The carotids, left middle cerebrals and anterior cerebrals are empty." On the right, "the upper surface of the caudate nucleus, in its anterior third, is found to be soft and of a rather hæmorrhagic appearance. There is no large blood-clot, however, and the blood seems to be mingled with the softened brain tissue. On section through the brain this softened area appears to be about 3 cm. in lateral diameter and to extend for 7-8 cm. antero-posteriorly, involving the caudate nucleus and the tracts just outside."

CASE XI. *Gonorrhœa. Vegetative and ulcerative endocarditis of pulmonic valves. Acute nephritis. Gonococcus obtained in pure culture from circulating blood.*

G. R., (Surgical, 35320; Path., 4222.) a man of 27 with no history of gonorrhœa, began, about 7 months before entry, to suffer from general weakness, submaxillary swelling on the left side, fever, abdominal pains and, a week later, chills recurring at irregular intervals. Blood cultures at this time were negative. Fever, irregular and intermittent, and chills persisted. There was a leucocytosis of from 10 to 15,000. Albuminuria and cylindruria. Petechial eruption.

A slight improvement during the summer was followed by return of active symptoms two weeks before entry on 17/IX/14. In the hospital he was pale and somewhat emaciated. The heart was a little large. "Over the apex the first sound is sharply replaced by a soft blowing murmur which can just be heard in the anterior axillary line but is much louder and more distinct over the second and third intercostal spaces just at the left sternal margin."

Nov. 2. "Over the præcordium a soft diastolic murmur can be heard; this murmur is sharply localized to the area over which

the systolic murmur was heard on admission and follows the latter murmur, replacing the second heart sound."

Spleen, large and palpable. Liver, 2 cm. below the costal margin. Slight tibial œdema. Petechiæ on different parts of the body. Leucocytes on entry but 8,000, rapidly rising.

Urine showed much albumin; hyaline, granular and cellular casts and numerous red blood corpuscles in the sediment. Phthalin, 13/IX, 41%; 18/IX, 34%.

Repeated cultures from the blood showed numerous colonies of characteristic gonococci growing only on blood agar plates.

The anæmia increased. There was tenderness over the spleen. Transfusions gave no permanent relief. There was an annoying diarrhœa. The blood pressure generally varied from 110/70 to 105/60, rising somewhat after the transfusions. On one occasion it fell as low as 80/40.

Death, 11/XI/14, more than 8 months after the onset of his symptoms.

At *necropsy* there was found a chronic urethritis, cystitis, orchitis, pyelitis. Numerous minute ecchymoses in the epicardium. The pulmonary valve showed large vegetations which were friable and definitely attached to the edges of the valve; the valve substance itself was apparently eroded in places where the vegetations were not present and the free edges of the valve were rolled and thickened. Acute hæmorrhagic nephritis. Central necroses in the liver. There was no note in the autopsy records as to cultures *post mortem*, but the pathological diagnosis was: "Gonococcus septicæmia and endocarditis."

CASE XII. *Gonorrhœa. Chronic urethritis. Prostatitis. Vegetative and ulcerative endocarditis of aortic valve. Cultures from the blood during life, negative.*

H. P., (Med., 42732; Path., 6061) a coloured laborer, aged 23, three weeks after the onset of a gonorrhœa which he thought had cleared up, began to suffer from severe headaches followed, a week later, by smothering sensations at night. There was diarrhœa and some slight stiffness of his joints.

On entering the hospital (11/XI/19) he was a healthy looking young coloured man with a temperature of 103° and a rather rapid pulse. The heart was a little enlarged; there was a soft, blowing diastolic murmur along the left sternal border and in the aortic area, and, at the apex, a rumble in diastole ending in a loud, ringing first sound. The pulse was collapsing. Neither liver nor spleen was felt. There was a leucocytosis of 19,760. The fever was remittent, between 100.8° and 103.8°. The urine showed a trace of albumin; hyaline casts and an occasional red blood corpuscle in the sediment. Spleen, not palpable. Cultures from the blood and urine were negative. Spinal puncture was negative. On the 15/XI he had a sharp, shaking chill. The patient failed steadily. The respiration became rapid and shallow. Signs of broncho-pneumonia appeared, and on 18/XI he died.

At *necropsy* there was found a chronic urethritis and prostatitis. On the mesial cusp of the aortic valve and ascending into the sinus of Valsalva behind it was a large soft pink friable vegetation; the cusps of the valve had almost been eroded away and leading from the sinus of Valsalva was a deep ulceration extending through the base of the aorta into the tissue of the right auricle and ventricle; this ulcerated excavation was filled with recent thrombus and formed a bulging mass partially occluding the tricuspid valve which was adherent to the protrusion behind it. The chordæ tendinæ of the tricuspid were somewhat shortened and thickened.

There were small areas of lobular pneumonia in the lower lobe of the right lung.

Most unfortunately, owing to a misunderstanding, no bacteriological examinations were made at necropsy.

CASE XIII. *Gonorrhœa. Arthritis. Irregular remittent and intermittent fever with chills. Cardiac enlargement with evi-*

dence of mitral disease. Splenic enlargement. Leucocytosis. Pleuro-pericardial friction. Cultures from circulating blood, negative. Death seven weeks after onset. Necropsy refused.

W. W. R., (Med., 7505) aged 32. Gonorrhœa, eight weeks before entry, 26/VI/1897. Three weeks later, arthritis of the hip and later of the knees. Two weeks before entry, severe headaches, pain in the back, chills, fever, sweating, nose bleed, frequent vomiting, slight cough. Has been in bed since the onset. On entry he was febrile; the pulse was accelerated; the heart was slightly enlarged. The first sound at the apex was replaced by a loud blowing systolic murmur heard throughout the axilla.

Second pulmonic, a little accentuated. Spleen palpable. Five years before, on a previous entry, the heart had shown no abnormalities. There was a purulent urethral discharge containing diplococci. Leucocytes, 21,000. There was high, irregular, remittent fever. The leucocytes rose to 23,000. The first heart sound became loud and very snapping; the murmur changed considerably in character from day to day. A pleuro-pericardial friction rub appeared. The patient steadily lost ground and died on the 2/VIII, seven weeks after the onset of his symptoms which were obviously those of an acute ulcerative endocarditis.

Necropsy, refused.

POST-ENCEPHALITIC BEHAVIOR DISORDERS IN CHILDREN

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For the past year, we have had an opportunity to study 11 children who have been brought to the clinic because of decided, profound changes in character and behavior. In all the cases, the disposition of the child had changed so suddenly and completely, or else been so much altered from its former behavior, that one could not avoid the conclusion that a specific etiological agent was at work. In each of the 11 cases, we have discovered that the change followed an attack of epidemic encephalitis.* From time to time our feeling that the connection between these alterations and encephalitis was genuine has been strengthened by the appearance of reports in the literature of several similar observations. Happ and Blackfan,¹ Findlay and Shiskin,² Grossman,⁴ and Francioni,⁵ have either noted the change in passing or have mentioned it specifically. Leahy and Sands³ report six cases to illustrate such a change. We are adding 11 cases to the literature to emphasize the point that the condition is apparently not uncommon, and because we believe that sick children are very apt to be classed merely as bad children and treated accordingly. In addition, certain reactions have been observed which are usually classified as purely functional or psychobiological, which apparently are closely related with the organic alteration. I refer especially to tics and excessive talk. While the main point of the inquiry has been directed toward the behavior changes, we have also noted the changes in sleep, the behavior during the sleepless periods, and the tics and excessive talk already mentioned.

In reporting the case records, we shall omit the details of the original febrile period, giving only enough facts to prove the reliability of the diagnosis. We have tried, when possible, to discover what rôle constitutional factors play in the etiology of the later changes. We have accordingly studied the family history for mental disturbances, and the individual's for neurotic traits (night terrors, enuresis, thumb-sucking, tantrums, and emotional insta-

bility). Our conclusions are not decisive, but the rôle of constitutional factors is certainly not great. Contrary to the belief of Francioni,⁵ who found a neuropathic background in all his cases, only two of the 11 children had shown any abnormality before the development of the infection, and in these cases the deviation was only slight—one child was described as having been "stubborn," and another as "more nervous than the other children." The parents of three of the children were nervous or psychotic. There also seems to be little relation between neuropathic background and chance of improvement, as two of the five improved patients had this background. We have thought that the reaction, instead of being due to a specific psychopathic background, might be explainable in terms of a release of a more primitive, instinctive reaction, such as the child shows who is undisciplined and untrained, that is to say, the reaction released is a more general childlike lack of control with no training inhibitions.

Behavior and Character Changes.—The most striking feature of these cases has been the changes in the character and behavior of these children. They became irritable, fussy, quick-tempered, or boisterous and restless. Instead of being able to play peacefully with other children as formerly, they became quarrelsome, teased the other children unmercifully, or showed streaks of brutality. At school, the teachers found them impudent, disrespectful, disobedient, or no longer amenable to discipline. This same disrespectful attitude was also displayed at home; they would curse their parents, or even strike at them. Instead of being interested in the school work and averagely diligent, they became indolent and indifferent. Several of the patients asserted their independence, insisting that they would do what they pleased. One little girl announced that she was now her "own boss." Staying on the street at all hours, and running away from home have been frequent. Some of the patients lost respect for personal and public property: they destroyed their own belongings or the house furnishings.

* The summary of the findings in each case is appended to show on what grounds the diagnosis was made.

They were frequently negligent about their personal appearance, slovenly, taking off their clothes in public, and were careless with their excreta, in some cases even playing with them. One of our children smeared with feces the walls and the face of her brother. Several have shown exhibition tendencies (showing the legs, leaving the trousers open, etc.), and one observer³ reports extraordinary sexual precocity in a boy of 8½, who attempted intercourse with his mother. Not infrequently patients were found fabricating or lying. The temper tantrums sometimes became so dangerous that everyone in the vicinity was in danger. Others had screaming attacks which were uncontrollable. Some of the children became emotionally quite unstable, weeping on the slightest provocation, were capriciously moody, or very irritable, fussy, whiny, and excitable. Discipline was simply resented and was quite ineffectual.

Grossman⁴ reports one interesting case, in which this order of events was reversed. A very unruly child became docile and well-behaved following the encephalitic attack.

Sleep Alteration.—The sleep alteration, as described by various observers,^{1 to 9} is strikingly uniform. The children found it impossible to go to sleep when they were put to bed, but generally would stay asleep very late on the following day. Waking the children early in the morning, or trying to tire them through strenuous exercise, did not aid them in getting to sleep the following night. Sedatives, in the cases we have had an opportunity to observe, have had a variable effect. At times a dose of barbitol or paraldehyde would compel sleep immediately, whereas on the very next occasion it would prove useless. We cannot subscribe to Rüttimeyer's⁹ conclusion that "the insomnia yields to psychotherapy unless there is evidence of persisting organic lesions." Psychotherapy has been entirely unavailing in our hands, although a number of our children showed no persisting signs of organic lesion.

The behavior during the sleepless periods has been characteristic. The child, as soon as it went to bed, would begin to be very restless, uneasy, and talkative. The talk would be continuous, but show no distortions. In some cases, the restlessness took the form of compulsive movements, of which we shall speak later. At times, the patients would sing or whistle, or become playful and prankish.

Over-talkativeness.—While over-talkativeness during the nocturnal insomnia has been quite common, a few of the children have shown the same feature during the daytime. This push of talk without distortion has been observed in adults,¹⁰ and has been recorded in children by several observers, although not specifically noted^{1, 2, 3}. Only one of our patients showed this symptom (Case 7).

Tics.—The compulsive, purposive, habitual movements at first impressed one as indistinguishable from the usual

tics which have been so uniformly explained as purely functional. This point of view has not been accepted by all observers, however. Adolf Meyer has repeatedly expressed the view that in stammering, in addition to the emotional factors involved, there was also a defect of the actual speech mechanism.

It would seem that the frequency of occurrence of these movements in this disease must point to some uniform organic basis behind the activity. What this is, we do not know. The following is the list of obsessive movements observed in the different cases: spitting, nose picking, lip picking, forced respiratory movements, touching the shoe and putting the hand in the mouth, wetting the finger and rubbing the cheek and ear with it, shaking the hand, making the sign of the cross, smoothing the hair, making signals, clicking movements of the tongue, clearing the throat, stretching the arms laterally in rhythms of some multiple of three, nose wiping movements, and others. Of these the spitting was probably the most common.

Therapy.—We have discovered nothing that is of any value in the treatment of these patients. Time seems to be the sole healing factor. The problems presented are at times very serious, as it is often impossible to maintain the children in their home environment, because of the behavior difficulties. Some of the children have been sent to hospitals for the insane, others to schools for incorrigible children. It has been the regular experience that *punishment helps not at all*. Nevertheless, we have persisted in our recommendations of a moderately rigorous regime, since we found that too lax treatment might tempt the child to use "doctor's orders," as an excuse for disobedience or misbehavior.

Prognosis.—The earlier reports give very guarded or bad prognostications. Our experience has made us more hopeful. We have seen complete recovery in 2 cases after 9 months and 3 years; marked continuous improvement in 4 cases after 6 months, 1½ years and 2 years, and 2 years; slight improvement in 3 cases after 3 years, 1¾ years, and 1½ years; and no improvement in 2 cases both with a duration of 1½ years. Over half of our patients, then, have shown marked improvement or recovery.

CASE REPORTS

CASE 1.—D. D. The patient, a colored girl of 11, was brought to the clinic because since March 1st, 1920, after an attack of epidemic encephalitis, she had become utterly unmanageable at home and in school. Although a somewhat stubborn child, she was not regarded as abnormal. Since her illness she had done very poorly at school because she would neglect her work for idle reading. When she was crossed in any way she would become very disrespectful to her mother and teacher, and if the children at school offended her, she knocked them down. It had been found necessary to remove her from two schools because of her cruelty to the other children. At nights she was very restless, and would talk and thrash about in her sleep.

She had to be committed to a hospital for the insane. A report later describes her as much improved.

CASE 2.—D. C. The patient was a white girl of 13½, who was brought to us on March 9, 1921, because of change in disposition, irritability, and quick temper, following an attack of epidemic encephalitis, in Dec. 1918. Her convalescence was protracted (4 montes) and during this period her mother noticed that the child was fussy and irritable, noisy, boisterous, and sang loudly. Finally she had to be removed from school because she idled and fought constantly with the other children. In June she became much worse, cursing her mother, and finally ran away from home. She became slovenly about her person, and careless about her excreta. Recently she had improved in the latter respect. She slept very poorly and would talk most of the night.

She had finally to be sent to a disciplinary school.

CASE 3.—W. S. The patient, a white boy of 13, was brought to the clinic Feb. 23rd, 1921, because of restlessness and sleeplessness, following an attack of epidemic encephalitis in April, 1920. Since the attack the boy has no longer been friendly and outgoing, but wants to sit by himself and read. He takes no interest in playing with the other children. At nights he is sleepless and restless, and as soon as he gets into bed, he begins to spit, picks his nose, and complains that he cannot get his breath. He does not go to sleep until one o'clock in the morning and then sleeps until noon the next day. Waking him earlier in the morning does not improve his sleep at nights.

Recently he has shown marked general improvement.

CASE 4.—L. R. A white boy, aged 9, was brought to the clinic, Sept. 30, 1921, because of nervousness, screaming spells and irritability, following an attack of epidemic encephalitis in Sept., 1919. Since his 3 weeks' acute illness, he has had uncontrollable screaming spells both at night and in the daytime. He has become very nervous and irritable, and will not be disciplined. Punishment does not seem to influence his behavior. In Jan., 1920, he began the habit of touching his shoe and putting his hand in his mouth, in a tic-like manner.

Recently he has begun to show improvement in all his activities.

CASE 5.—A. M. A white boy, aged 8, was brought to the clinic Oct. 11th, 1920, because of sleeplessness, and "funny talking" at night, which came on following an attack of epidemic encephalitis in Jan., 1920. After the acute infection the boy became very restless when put to bed. He began to puff and then scream, wept, ran from room to room, tearing at his clothes, etc. He could not get to sleep until very late and then went to sleep the following day in school. He preferred to stay at home and "play in water." He fought with the other children, refused to obey his mother, and was generally impudent. He became careless about his clothes and allowed his trousers to remain unfastened. He drooled saliva, spit constantly, and chewed his handkerchief.

His behavior has improved somewhat lately, but not much.

CASE 6.—A. S. A white girl, aged 8 years, was brought to the clinic Jan. 8th, 1921, for "restlessness at night," following an attack of acute epidemic encephalitis in June, 1920. After the first month of the illness she began to sleep very little at night and was very difficult to arouse in the morning. In Oct., 1920, she began to wet the bed at night and her mother found her sitting up in bed "breathing hard." In Sept., 1921, the child was brought to the clinic again because she had become very destructive, tearing up her copy-books, pulling off the wall-paper, and throwing water around the house. Two weeks before we saw her she had smeared the wall, the bed, and her brothers' faces with feces. At school she is reported to be using "bad language" and cursing. She shows violent temper tantrums and has announced that she is her own boss. She can no longer play with other children, because she quarrels with them and abuses them. Punishment avails nothing. All this is all a total reversal of her former behavior. She was a very healthy, affectionate child and was very obedient.

Up to date no improvement has been reported.

CASE 7.—G. R. A colored girl, 13 years of age, was brought to the clinic on April 8, 1921, because of "absent-mindedness and no memory," following an attack of epidemic encephalitis in February, 1921. Immediately following the acute attack (two weeks), the patient would sit quietly by herself, making revolving motions with her hands and laughing in a silly manner to herself. She slept poorly and did not want to go to bed at night. She refused to obey her father and was very resentful to him. If he touched her, she got angry, and fought him. She had become very careless of her personal appearance, allowing her stockings to hang down, exposing her legs before boys, etc. She had no ambition to return to school. By June, 1921, the irritability and insomnia had disappeared, but she talked very little. It was noticed that she picked her nose and lips excessively. Her appetite had become huge. In August, she began to talk more and more, until by September she was over-talkative and very over-active and playful, teasing her mother and father.

In October, 1921, she was practically her old self.

CASE 8.—K. S. A white boy, of 14 years, was brought to the clinic September 29, 1920, because of a "lump in the throat and constant spitting," following an attack of epidemic encephalitis in February, 1920. He had slept constantly during March, but in April he became sleepless and restless, talking incessantly at night. He began to spit obsessively and complained of a lump in the throat. Then appeared various tic-like movements:—wetting his fingers and then rubbing his cheeks and ears. He would laugh very readily and without adequate cause.

Up to date, there has been no improvement in his behavior.

CASE 9.—M. W. A white boy, of 12, was brought to the clinic March 16, 1921, because of "incurability" following an attack of epidemic encephalitis in January, 1919. The following spring and summer he was very sleepy all the time, spent most of his time lying about the house, and wept over almost anything. Since the summer of 1919, he has been very restless and feels he must be in motion constantly and, if hindered, becomes fussy and whiny. He became incorrigible at school, was impudent, "talked back," and was very resentful over discipline. He played truant frequently, and on several occasions stayed away from home all night. His mother hoped that discipline would help him, and had him placed as a ward of the juvenile court. However, neither this nor the application of corporal punishment helped. In August, 1920, he began to show some improvement, and was better behaved at home. At present, he is still more improved, and is doing very well at school, having been promoted twice. His I. Q. at the first examination was 87; it is now 100.

CASE 10.—T. C. A white boy, 9 years old, was admitted to the clinic June 12, 1920, from the Harriet Lane Home for Invalid Children, because of restlessness following an attack of epidemic encephalitis in October, 1919. Following the pyrexial attack, he began to sing and whistle constantly, became unruly, refused to eat and made odd movements with his hands and legs. During January, 1920, the patient would refuse to stay in bed, and wanted to be on the street all night. He removed his clothing while on the street, but replaced it when told to do so. His restlessness and irritability increased, and he was brought to the hospital. In the hospital he showed very odd restless movements, which he would repeat over and over, crossing himself, smoothing his hair, making signals, and queer clicking movements with his tongue. Nothing could control these movements. He sang and whistled all the time. He slept very badly and was very irritable and capriciously moody.

Up to August, 1921, there had been no change in his condition.

CASE 11.—P. E. A white boy, 10 years old, was admitted to the clinic May 19, 1920, because of restlessness, sleeplessness and stereotyped movements, following an attack of epidemic encephalitis in February, 1920. The boy made what appeared to be an excellent recovery from the acute attack in 3 weeks, although he complained of a fullness in the throat. Soon it was noticed that he was "nervous," fidgety, and could not sleep at night, although he slept well in the day time. He talked all night, but all his talk was sensible. He began to repeat certain complicated movements over and over. (1) He would take a deep breath, then expel it with a short cough. (2) He would stretch out his arms two or three times in rapid succession. The leg also was used at times. These movements seemed to relieve some indefinite discomfort. (3) He put his finger to his nose, with a wiping movement. These movements were repeated constantly with a rhythmicity of three or a multiple of three. While in the hospital here, he demanded constant attention, was very restless, and questioned everything that was done for him. He quarrelled frequently with another child on the ward. He would weep on the slightest provocation. While in the clinic, he developed a spitting tic. His sleep at night was very poor. Previous to the onset of his illness, he had been entirely normal.

On discharge, July 4, 1920, he was decidedly improved.

CONCLUSION

1. Definite character changes are sequelæ of epidemic encephalitis.
2. The alterations of sleep and the activity during wakefulness are characteristic.
3. Tics have been so frequently observed as to warrant the search for a structural background.
4. Therapy seems to be useless.
5. Over half of the reported cases have improved.

DATA UPON WHICH THE DIAGNOSIS WAS BASED

CASE 1. Fever, ptosis, and typical spinal fluid alterations.

- 2. Fever, diplopia, somnolence, left-sided weakness.
- 3. Fever, right internal strabismus, restlessness, sleeplessness, odd behavior with delirium.

- 4. "Flu," fever, delirium. Bi-lateral ptosis.
- 5. "Flu," with sleeplessness, double vision and fever.
- 6. Fatiguability, lethargy, fever, then sleeplessness.
- 7. Fever, delirium, drowsiness, headaches, nocturnal excitability.
- 8. Fever, drowsiness, constant sleep, Parkinsonian rigidity.
- 9. "Flu" with drowsiness, lethargy, and drowsiness.
- 10. Fever, insomnia, restlessness, spinal fluid findings typical.
- 11. Fever, delirium, muscular jerkings, insomnia, and typical spinal fluid findings.

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TOLERANCE AND ACQUIRED TOLERANCE OF THE MESENCHYME CELLS IN TISSUE-CULTURES FOR COPPER SULPHATE AND SODIUM ARSENITE

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Natural tolerance and acquired tolerance exhibited by an organism for toxic substances may depend upon the neutralization of the toxin by extracellular substances or upon some intracellular mechanism; that is to say, the toxin is either neutralized without or within the cells of the organism. In dealing with the entire organism it is impossible, in most instances at least, to determine whether the phenomenon is intracellular or extracellular. In tissue-cultures, on the other hand, as with some of the lower organisms, there is the possibility of testing directly the tolerance of cells for toxic

substances. Unicellular organisms tolerate certain poisons and even develop an acquired tolerance or immunity for concentrations above the lethal dose.

Davenport and Neal (1896)* found that *Stentor coeruleus*, a unicellular organism, after being kept for a few days in a weak solution of corrosive sublimate, was able to survive a stronger solution several times as

* Davenport, C. B. and H. V. Neal, 1895. Acclimatization of organisms to poisonous chemical substances. *Arch. f. Entwcklingsmech. d. Organ*, II, 564-583.

long as a control animal of the same series. Daniel (1909) ‡ found that certain strains of *Stentor coerulesus* and *Spirostomum ambiguum*, unicellular forms, after living for a few days in 1 per cent ethyl alcohol, had increased resistance to stronger solutions, as shown by the fact that the organisms were not killed so quickly in a lethal solution and that many continued to live in a stronger solution than that in which they could have lived before "acclimatization." One strain of *Stentor* did not develop an acquired tolerance. There was no observed development of acquired tolerance for hydrochloric acid or sodium hydroxide. With a weak solution of alcohol (1 per cent) the beginning of acclimatization (acquired tolerance) was usually evident within a few hours. This increased in a fairly uniform ratio until about the fourth day, at which time the maximum degree of immunity might be expected. Daniel found that a one per cent solution of the alcohol gave greater tolerance than a half per cent solution, while stronger solutions decreased the resistance by producing injury to the organism.

Concerning the ordinary embryonic mesenchyme cells of the chick embryo, the following experiments will show that they have a certain amount of tolerance for the two inorganic poisons used and that an increased or acquired tolerance can be produced by cultivation in weak solutions of these substances. In testing the degree of acquired tolerance, the cultures were washed with several drops of the solution in such a manner that the practically undiluted solution must have bathed the cells, and any neutralizing substances produced by the cells during their cultivation in the weak or immunizing solutions were entirely washed away.

The cultures were made in the usual manner from tissues of 7 to 11 day chick embryos. Locke-Lewis solution, namely 85 c.c. of Locke solution (NaCl .9%, CaCl_2 .024%, KCl .042%, NaHCO_3 .02%), 15 c.c. of chicken bouillon and 0.5 gm. of dextrose, was used. The observations were confined, for the most part, to the ordinary mesenchyme cells from the subcutaneous tissue and from the lungs. Stock solutions of copper sulphate and sodium arsenite in distilled water were added in appropriate amounts to the culture medium. Several series of cultures, varying from 5 to 10 in number, were made for each solution tested and at the same time about the same number of controls were made, using tissue from the same embryo.

The effects of the poisons were measured (1) by comparing the percentage of cultures which showed growth or migration, the size of the growth (in a rough way), and the maximal duration of life with the controls; (2) by comparing the time required to kill cells that were cultivated in these weak solutions with the time required

to kill the cells in the controls when treated with strong solutions. The first group gave some idea of the natural tolerance, the second the acquired tolerance.

* EXPERIMENTS WITH COPPER SULPHATE

With a 1 to 25,000 solution no growth or migration was obtained. In six series with a 1 to 50,000 solution, 50 to 80 per cent of the cultures, with an average of 66 per cent, showed migration. The controls averaged 90 per cent. The control cultures were larger. The maximum life of the copper sulphate cultures varied from 5 to 12 days with an average of 8 days, while the average for the control was 13 days. In seven series with a 1 to 100,000 solution, from 30 to 100 per cent grew, with an average of about 60 per cent. The controls averaged 94 per cent; they were larger, as a rule, than the copper sulphate cultures. The maximum duration of life of the copper sulphate series varied from 4 to 11 days, with an average of about 7 days. The maximum for the controls varied from 8 to 11 days.

In order to test the acquired tolerance, normal 2 and 3 day cultures were first stained for 10 minutes in a weak solution of neutral red and janus green and then washed with a 1 to 5,000 solution of copper sulphate in Locke's solution. It was found that cells lived from 17 to 38 minutes, averaging about 26 minutes. The death changes were characterized by coagulation and swelling, with increased visibility of the nucleus, which became sharply outlined by what appeared to be a nuclear membrane. Accompanying these nuclear changes was loss of the greenish color from the mitochondria and the red color from the degeneration granules. The nucleoli often became stained. After death the whole cell was sometimes diffusely stained. Accompanying all these changes, which began about 10 minutes after the application of the copper sulphate, was a vacuolization of the cytoplasm.

Cultures that were made at the same time with 1 to 100,000 copper sulphate in Locke-Lewis solution were treated in the same manner with a 1 to 5,000 solution of copper sulphate. The death changes were similar and the cells were considered dead in from 25 to 56 minutes. The average time was 42 minutes. As compared with the average time for the normal cultures of the same age, 26 minutes, this indicates that the cells acquired a considerable tolerance for the copper sulphate.

EXPERIMENTS WITH SODIUM ARSENITE

In the 1 to 50,000 solution of sodium arsenite there was no growth. In one series with 1 to 100,000 solution, about 50 per cent of the cultures showed migration as compared with 85 per cent of the controls. Their maximum life was 1 day as compared with 9 days for the controls. In two series with a 1 to 200,000 solution, about

‡ Daniel, T. F., 1909. Adaptation and immunity of lower organisms to ethyl alcohol. *J. Exper. Zool.*, VI, 571-611.

30 per cent showed migration as compared with 80 per cent for the controls. Their maximum life averaged 3 days as compared with 9 days for the controls. In seven series with a 1 to 250,000 solution, from 60 to 100 per cent of the cultures showed migration, with an average of 77 per cent, as compared with 90 per cent for the controls. The average maximum duration for the seven series was 6 days as compared with 8 days for the controls. In three series with a 1 to 500,000 solution, 88 per cent showed growth as compared with 86 per cent for the controls. Their average maximum life was 6 days as compared with 9 days for the controls.

To ascertain the acquired tolerance, normal 3 to 6 day cultures, after 10 minutes staining with a weak solution of janus green and neutral red, were washed in the usual

manner with a 1 to 10,000 solution of the sodium arsenite. Cell death occurred in from 22 to 50 minutes, averaging about 33 minutes. Cultures of the same age, growing in the 1 to 250,000 solution of sodium arsenite, showed cell death in from 39 to 70 minutes, averaging about 53 minutes. The death changes were somewhat similar to those in the copper sulphate experiments, except that the nucleus became shrunken and more highly refractive.

CONCLUSION

Embryonic mesenchyme cells, cultivated in weak solutions of copper sulphate and sodium arsenite, develop in the course of two days an acquired intracellular tolerance for strong doses of these two poisons.

NOTE ON THE ABDOMINAL CHROMAFFIN BODY IN DOGS

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In 1914 we² published the results of some experiments on the suprarenal glands in dogs, with especial reference to the functions of the interrenal portions, reaching the conclusion that it is the cortex rather than the medullary portion that is essential to life. In that series suprarenal insufficiency was produced by repeated surgical operations; during the past year we have tried to produce a suprarenal insufficiency by a combination of surgical removal and implantation of radium.[†]

Our previous experiences had shown that if half (and in some instances as little as one quarter) of one suprarenal gland is left in place, the remaining portion, together with the other gland, may be removed without seriously disturbing the health of the animal. During the past year another series of experiments was carried out. In these we removed one suprarenal gland and from one quarter to three quarters of the other, and at the operation implanted capillary glass points, containing radium emanations in varying amounts, into the portion of the gland that remained. From a total of 16 dogs treated in this manner 10 died, and this note is limited to the description of the changes observed in the abdominal chromaffin body in these 10 dogs.

The investigations of Stilling,⁷ Swale Vincent,⁸ and Kahn⁹ have shown that in the dog, as in many mammals, there is a conspicuous band of more or less continuous chromaffin tissue lying ventral to the abdominal aorta

and just beneath the peritoneum.* Vincent and Kahn have made careful anatomical and physiological investigations of this chromaffin body in the dog. It varies from 60 to 80 mm. in length and 2.5 to 5 mm. in width, is slightly less than 1 mm. in thickness, and weighs approximately 0.06 grams. The arteries supplying this body arise directly either from the aorta or the right internal spermatic; the veins empty into the right or left spermatic vein. The cells comprising it give the same brownish reaction with the salts of chromic acid that is so characteristic of the medullary portion of the adrenal. This brown stain is believed to be due to the interaction of the chromic salts with adrenalin. Kohn⁶ has pointed out, however, that the cells of the chromaffin body rarely stain as deeply as do the medullary cells of the adrenals.

The physiological researches of Biedl and Wiesel¹ Vincent,⁹ Fulk and MacLeod,⁴ and Kahn⁵ have demonstrated that a substance identical with the active principle of the suprarenal medulla, may be recovered from the abdominal chromaffin body. Biedl and Wiesel prepared extracts of the human accessory chromaffin tissue (organ

* Stilling was the first to show that the collections of cells closely resembling those of the adrenal medulla, which are associated with the sympathetic nervous system, give a reaction with chromic acid salts, similar to that of the medulla of the adrenal. He called these groups of cells "chromophil bodies." Vincent later changed the spelling to "chromaphil." Kohn⁶ introduced the term "chromaffin," and designated collections of these cells as "paraganglia." He directed attention to a conspicuous mass of these cells lying ventral to the abdominal aorta which was constantly present in the mammals investigated by him and which he termed the "paraganglion aorticum abdominale." In the subsequent literature this body has usually been referred to as the "abdominal chromaffin body."

† The radium used in these experiments was given us by the Howard A. Kelly Hospital.

of Zuckerkanndl) from new-borns, and found that such an extract has the same effect on the blood-pressure and heart-rate as suprarenalin. Vincent was able to demonstrate that an extract of the abdominal chromaffin body of a dog will produce a rise in blood-pressure when injected into the blood-stream of another dog. He concluded that all accessory chromaffin tissue contains adrenin, or a substance having similar chemical and pharmacodynamical properties. Fulk and MacLeod made physiological observations on a variety of animals and concluded therefrom that "acid extracts of the retroperitoneal chromophil tissue of man, the dog, the cat, the rabbit, the guinea-pig, the white rat, the calf, the sheep and the pig, have the same physiological action on intestinal and uterine muscle as the active principle of the medulla of the suprarenal glands." Kahn found that extracts of the medullary and of the accessory chromaffin tissue of the dog are identical, in that they both produce a rise in blood-pressure in the rabbit and cat, a mydriatic action in the frog, and a glycosuria following their intravenous injection in the dog. He showed, by accurate determinations of the respective weights of the dog's adrenal medulla and the abdominal chromaffin body, that the latter represents about one-fourth to one-tenth of the total weight of the former. He found, however, that, measured by pharmacological action, the retroperitoneal chromaffin body possesses only one-twelfth to one thirtieth as much active principle as the total adrenal medulla. Furthermore, a given quantity of medulla by weight contains three to four times as much active principle as an equal amount of the chromaffin body. That the chromaffin body contains the same active principle as the adrenal medulla seems to have been amply proved by the work of these investigators.

There remained, however, the question whether or not the abdominal chromaffin body is a functioning organ actually liberating its secretion into the blood-stream. This also Kahn attempted to answer. He collected blood from the veins of the organ, took the serum therefrom and tested its vasoconstrictor action in the hind-legs of a frog by the Trendelenburg method of perfusion, using blood-serum from other veins and the carotid artery as controls. He found a constant difference in respect to the vasoconstrictor action of the employed sera. The serum obtained from the veins of the paraganglia had a stronger and more sustained action than that from the carotid artery or other veins used as controls. Kahn attributes this difference to the presence of a larger amount of blood-pressure-raising substance in the paraganglion veins than in the control veins. That this substance is actually adrenalin seems highly probable from the fact that an increased amount of blood-pressure-raising substance could be demonstrated in the veins of this organ in which adrenalin has been proved beyond doubt to occur.

The method of procedure on the 10 dogs in which we studied the chromaffin reaction of the abdominal chromaffin body is herewith given in condensed tabular form:

TABLE

	Amount of Adrenal Gland left at Operation	Amount of Radium Implanted	Length of Survival
1. Dog 19	$\frac{1}{5}$ of 1 adrenal	1-4 6/10 mc. point	24 hours
2. Dog 22	$\frac{1}{2}$ of 1 adrenal	2-1 7/10 mc. point	24 hours
3. Dog 20	$\frac{1}{2}$ of 1 adrenal	2-1 7/10 mc. point	5 days
4. Dog 16	$\frac{1}{2}$ of 1 adrenal	1-4 6/10 mc. point	6 days
5. Dog 17	$\frac{1}{2}$ of 1 adrenal	2-4 6/10 mc. point	7 days
6. Dog 15	$\frac{1}{2}$ of 1 adrenal	1-4 6/10 mc. point	9 days
7. Dog 18	$\frac{3}{4}$ of 1 adrenal	3-4 6/10 mc. point	14 days
8. Dog 5	1 entire adrenal	2-7 mc. point	16 days
9. Dog 21	$\frac{1}{4}$ of 1 adrenal	1-1 7/10 mc. point	21 days
10. Dog 23	$\frac{1}{2}$ of 1 adrenal	1-1 7/10 mc. point	32 days

It will be observed that the adrenal glands were reduced by surgical extirpation to one or a fraction of one adrenal into which fragment radium emanation was then implanted. The animals died at intervals of 24 hours to 32 days following this procedure.

In every instance an autopsy was performed immediately after death. The retroperitoneal tissue suspected of containing the abdominal chromaffin bodies was removed, washed free of blood, and immersed immediately in a mixture of 9 parts of 3.5 per cent potassium bichromate and 1 part of 40 per cent formaldehyde (Kohn's fluid). At the end of 3 hours the tissue was rinsed in tap-water and transferred to 10 per cent formalin for 24 hours for further fixation. It was then washed thoroughly, passed into 60 per cent alcohol and in this latter examined for chromaffin bodies. A small piece of the principal chromaffin body was removed for paraffin imbedding and sectioning, while the bulk of the tissue was bleached in hydrogen peroxide in the sunlight (95 per cent alcohol, 7 parts; water, 1 part; hydrogen peroxide U. S. P., 3 parts) for further demonstration of the microscopically visible chromaffin bodies.

Examination of the abdominal chromaffin bodies in every instance showed a well-marked chromaffin reaction, approximating the color intensity observed in normal animals and indeed occasionally somewhat darker; in no instance was it less intense than in normal animals. No hypertrophy, however, in the sense of an actual increase in number or size of the chromaffin bodies, could be ascertained, either macroscopically or microscopically.

In each instance, histological sections of the principal chromaffin body showed well-preserved cells containing in their cytoplasm an abundance of chromaffin material. Although every section exhibited a typical and well-marked reaction, the picture was not always uniform. In the majority of animals, each cell showed a dark brown reaction, the chromaffin substance completely filling the cytoplasm and occasionally obscuring the nucleus. In a

lesser number, areas were seen in which it was unequally distributed, some cells exhibiting a very deep reaction, while neighboring cells contained clear cytoplasmic vacuoles of various sizes. Such differences in the staining reaction are commonly encountered in the normal adrenal medulla and hence may be attributed to different states of activity on the part of normal cells.

We have found, then, that in dogs in which the adrenals have been destroyed the abdominal chromaffin body at the time of death exhibits a normal or even more intense chromaffin reaction, indicating normal or slightly increased activity on the part of this tissue. Further, we have learned from Kohn that the principal chromaffin body represents one-fourth to one-tenth of the total medullary tissue, but possesses only from one-twelfth to one-thirtieth as much active principle. The problem now arises whether or not this fraction of the total chromaffin tissue represented by the abdominal chromaffin body is capable, in adrenalectomized dogs, of supplying that portion of the function of the adrenal glands subserved by the medulla? While we believe that this question has not been definitely solved, several facts may be cited that tend towards an affirmative answer. One of these is that, at the time of death, the abdominal chromaffin body is found replete with secretion, instead of depleted. However, it cannot be ignored that the chromaffin body could conceivably be found at death to be in a fully functioning state without having fulfilled the minimum requirements necessary to maintain the functional activity of the muscular and vascular apparatus, if, indeed, the secretion of chromaffin tissue contributes to any such important function. Another fact that tends to prove the ability of the chromaffin body to supply the physiological demand for epinephrin is the observation, repeatedly made on other ductless glands (the thyroid, parathyroids, islets of Langerhans, and testes), that only a small fraction of the entire organ is necessary to fulfill the function of the whole.

Perhaps the strongest evidence that the abdominal chromaffin body acts as a substitute for the adrenal medulla is the observations by Biedl,² and by the authors,³ that dogs survive extirpation of the adrenals provided a portion of the cortex only remains.

Is there any evidence as to the relative importance of the cortex and medulla in maintaining life? It seems to us that there is conclusive proof that the cortex is necessary to life, for when, in the above mentioned experiments, the remaining fragment of cortex was removed the animals died. The same experiments prove that the adrenal medulla is not necessary to life, though they by no means demonstrate the same for the entire chromaffin system. The decision that any part of the chromaffin system is or is not necessary to life must rest upon experiments in which the abdominal chromaffin body is removed from animals in which the adrenals have previously been reduced to a cortical fragment. If the animals survive, the necessity of the chromaffin tissue to the maintenance of life could be questioned. It would seem only remotely possible that the insignificant, scattered groups of chromaffin cells remaining would be capable of supplying the function of the chromaffin system. That removal of the abdominal chromaffin body alone, without disturbing the adrenals, is symptomless, has been demonstrated by Kahn. There is great likelihood that the fall in blood-pressure, which is a characteristic symptom of adrenal extirpation, has its origin in the removal of the cortex.

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NOTES ON NEW BOOKS

The Heart, Old and New Views. By H. L. FLINT. Cloth, \$4.00. (New York, Paul B. Hoeber, 1921.)

Among the many shorter texts dealing with the cardiac irregularities we know of none which presents the subject in the interesting way so successfully attained by the author of this volume.

Written with the two-fold purpose of appealing to those who may have but little time to sift critically the exhaustive literature, as well as for those who desire to turn to the original sources, its value is greatly enhanced by the historical treatment of the subject and by the marginal references to almost all, if not all, the really important literature. The first part of the volume dealing with the heart in antiquity comprises a delight-

ful review of the knowledge of the time of Hippocrates, Galen and Vesalius, and concludes with a study of the period beginning with the discovery of the circulation by Harvey down to recent times. It includes such names as Malpighi, Loeuwenhook, Von Helmont, Descartes and Stenson, and among a host of other equally conspicuous investigators reference is made to the work of James Keill (1673-1719), who was among the first to apply mathematics to the problems of circulatory hydraulics. Part II takes up the development of instruments and instrumental methods, and here again the author is equally successful in his critical analysis and in his appreciation of the fundamental importance of physiology.

The various methods for determining the blood pressure, blood

velocity and output of the heart are referred to briefly. The use of the clinical polygraph, the physiology of the venous curves and the interpretation of the polygraphic records are dealt with in detail, while the subject of electrophysiology and the interpretation of the normal galvanometric curves is soundly discussed and lucidly described. The chapter devoted to a consideration of the five functions of the heart muscle is a concise and clear presentation of the modern conception of cardiac physiology as originally enunciated by Gaskell, and presents a very clear analysis of the much discussed doctrines dealing with the neurogenic and myogenic theories of cardiac function. If we were to venture any criticism of the author's treatment of this phase of the subject, it would be that we fear lest, by the very necessity of the method followed in his analysis of the various functions of cardiac muscle, he has conveyed the impression of an apparent interdependence of physiological function which actually does not exist to the degree suggested by the text.

The cardiac irregularities are discussed in a very satisfactory way and are well illustrated by both polygraphic and electrocardiographic curves, a large number being taken, as the author states, from the writings of Lewis and Mackenzie, to which are added many of his own records.

Short chapters on the systolic murmur and the principles of treatment conclude the volume, the value of which is not a little enhanced by a most satisfactory index.

This is a work which it is a genuine pleasure to commend, quite as much for the way in which the subject matter is presented as for the soundness of the views expressed. It is interesting, as illustrating the rapid advances of our knowledge, that even in this book, though bearing the date of 1921, there is no allusion to the modern theory of the "circular movement" in the description of auricular flutter and fibrillation.

We are confident that a second edition will soon be called for in which the more recent contributions of Lewis and his co-workers will be incorporated; and may we venture to add the hope that reference to the work of Einthoven and Hugenholz and to that of Arbeiter, in connection with the subject of electrical and mechanical phenomena, may find a place in any subsequent revision.

E. P. C.

Tuberculosis in Infancy and Childhood. By J. CLAXTON GITTINGS, M. D., FRANK CROZER KNOWLES, M. D. and ASTLEY P. D. ASHURST, M. D. Cloth, \$5.00. (Philadelphia and London, J. B. Lippincott Company, 1922.)

The authors approach the subject of tuberculosis from the point of view of the pediatrician. In most text-books on pediatrics, a proportionately large space is devoted to tuberculosis, but in such books the really detailed information that is often required is apt to be missing. This volume fills a definite need in furnishing such information in a concise but comprehensive manner, and in presenting impartially a summary of our present knowledge of tuberculosis, as it occurs in infancy and childhood. In several respects it illustrates the changing attitude of pediatricians in regard to the prognosis in tuberculosis, in pointing out that the mortality in infancy, although very high, is distinctly lower than was formerly believed. This change in attitude is due largely to the increased use of well defined methods of diagnosis, chiefly the intradermal tuberculin test. The chapter on the use of Tuberculin in diagnosis is particularly well presented. The authors stress the point to which all pediatricians bear witness; namely, that until in the most advanced stages tuberculosis in infants often does not interfere noticeably with the general nutrition.

The book is well arranged. The few illustrations are very good, but add little to its value.

B. T.

The Blood Supply to the Heart in its Anatomical and Clinical Aspects. By LOUIS GROSS, M. D., C. M. Cloth, \$5.00. (Paul B. Hoeber, New York, 1921.)

This monograph presenting the results of the author's researches upon the structural evolution of the circulation of the heart, based upon an exhaustive study of one hundred hearts of all age periods, is a very valuable contribution, and illustrates in a striking way the important clinical application of a study primarily undertaken as a purely anatomical investigation. After describing briefly the technical methods used by previous investigators, the author discusses the difficulties involved in any attempt to inject the coronary circulation, and describes in detail the technique followed in his method of procedure. He then discusses the vascular supply to the auricles and ventricles and summarizes the circulation to the heart as a whole, emphasizing the fact that, although there can be no hard and fast line of demarcation between the distribution of the right and left coronary arteries, which, as he has shown, have profuse and abundant anastomoses, it is quite possible to adopt an arbitrary division, with certain overlapping territory, which permits of an accurate description of the distribution of the coronary circulation in the average heart. He classifies by means of tables and text all the most important deviations from the usual in his series of hearts.

We have been particularly interested in the account of the blood supply to the neuro-muscular tissue, and have been greatly impressed by the evidence adduced to show that there exists a specific vascular supply for the sino-auricular node from the *Ramus ostii cave superioris* of the right coronary—occasionally arising from the left—and that there is no evidence for the opinion that anastomoses exist between the vessels supplying the sino-auricular and the auriculo-ventricular nodes; a view entirely confirming and amplifying the previous observations of Keith and Flack and of Koch. The author is of the opinion that a distinct and specific blood supply exists for both the sino-auricular and auriculo-ventricular nodes, the main stem of the His bundle, the first portion of the left branch and a large part of the right branch of the specialized conduction tissue—an anatomical difference of the greatest practical significance in our interpretation of lesions arising from any functional disturbance due to changes in the vascular supply. This is further emphasized by a brief discussion of a few instances from the series of cases reported by Moenckeberg. No more appealing brief for the necessity of an accurate correlation of our clinical and galvanometric observations with careful anatomical and injection studies could be offered than this chapter upon the blood supply to the neuro-muscular system. The all too ready assumption of *post hoc propter hoc* plays often far too large a part in many utterly uncontrolled conclusions.

The question of the blood supply to the heart valves has been without doubt one of the most controversial fields of cardiac vascular morphology. Dividing the prevailing conflicting evidence into three main groups, the author concludes, as a result of experimental work and his interpretation of the literature, that each of these conflicting views may be in a measure correct, if not accepted in entirety, and that they can all be coordinated into a logical and reasonable explanation for the genesis of many cases of acute valvular endocarditis. In this connection the following paragraph may be quoted in full:

"If the literature on the mechanism of acute valvular endocarditis is now reviewed in this light, one is at once struck with the fact that the occurrence of endocarditis bears a strikingly close relationship to that of the existence of musculature and of blood vessels in valves. This becomes even more striking when the incidence of endocarditis is considered from the point of view of the frequency with which it occurs on the left and right sides as well as on the individual valves and cusps."

The author's premises in this connection based upon his experimental data, as given on page 71, are very interesting and suggestive, and impress one as being sound. We wish that we might quote at length from his discussion of the subject of anastomoses between the coronary arteries, but this chapter must be read in its entirety to be justly appreciated. His general conclusion that the heart is, perhaps, the richest organ in the body as regards capillary and precapillary anastomoses between branches of the same coronary artery, as well as between branches from both coronaries, seems to be abundantly supported on the basis of his available evidence and exhaustive study, and can but reinforce our conception of the marvellous provision for the maintenance of the normal anabolic and katabolic functions.

We know of no text which can in any sense be said to approach the careful detailed and concise outline covered by the author in his description of the venous circulation of the heart. The concluding chapter, dealing with the age period changes in the blood supply and their pathogenic relations, furnishes a most stimulating and suggestive discussion based upon a broad biological consideration of all the factors at work in the postnatal evolution of the individual. The volume is illustrated throughout with really remarkable plates and its value as a work of reference is tremendously increased by the exhaustive bibliography and by an index of authors, as well as an unusually satisfactory index of subjects.

E. P. C.

Obstetrical Nursing. A Text-book on the Nursing Care of the Expectant Mother, the Woman in Labor, the Young Mother and Her Baby. By CAROLYN CONANT VAN BLARCOM, R.N. (The Macmillan Company, New York, N. Y.) (\$3.00)

Examination of many of the text-books of obstetrics for nurses shows that most of them present the subject inadequately and make little attempt to elevate the art of midwifery to the science of obstetrics. Some are so antiquated and scientifically inaccurate that they are of little value. In many instances the attempts at brevity so limit description of the methods employed in the various clinics as to restrict their use to the hospitals from which they originated.

It is a pleasure to welcome a book which has overcome most of these objections. Miss Van Blarcom takes the stand that the obstetrical nurse is more useful if she understands more than the nursing care of her patients. Therefore, the anatomy, physiology and pathology of pregnancy, labor, the puerperium and the newborn child are adequately and simply explained. The author has familiarized herself with the obstetrical procedures of the leading hospitals of this country and Canada, and this broad view is reflected in her book. It gives the nurse a much clearer understanding of the subject and prepares her for the varying practices with which she may meet in her work. The nursing care of the mother and child is presented in a manner quite different from usual. While the various procedures employed in the better hospitals are described in detail, it is recognized that many women are delivered at home and that the nurse must often work with inadequate equipment. To meet this need, therefore, the author has simplified, as far as possible, the routine hospital procedures.

The author is to be commended especially for including in her book many of the modern ideas concerning prenatal care, the mental hygiene of the expectant mother and the nutrition of the mother and child. These chapters are among the best in the book and add much to its value. Incidentally, much of this subject matter might well be incorporated in the texts for medical students and practitioners.

Throughout the book sustained effort is made to impress the nurse with the fact that she is dealing with an individual rather than with a "case," that her patient is going through the most eventful period of her life, many of whose phenomena she does

not understand, and that thoughtful sympathy on the part of the nurse is as much her duty as the actual nursing care of the patient.

The book is written in an interesting and simple style, and the text is clarified by numerous excellent photographs, drawings and charts. It adequately fills a real need in the teaching and training of nurses.

J. W. H.

Management of the Sick Infant. By LANGLEY PORTER, M.D. and WILLIAM E. CARTER, M.D. Cloth, \$7.50. (St. Louis, C. V. Mosby and Company, 1922.)

The authors have prepared a book that deals exclusively with the management of diseases peculiar to children and infants. The commoner symptoms of disease, such as convulsions, vomiting and so forth, their causes and methods of treatment are taken up in order. Following this, the disease entities of the different systems are similarly treated. At the end of the book there is a long list of prescriptions, which the authors have found useful. The chapter on the preparation of various food substances is particularly well arranged and should prove very helpful. The book is of use particularly to the practicing pediatrician and should be of value as a book of reference for any physician in his management of sick children.

There is nothing revolutionary or startlingly new in the volume. The authors have presented, in an exceptionally complete way, detailed directions for the management of all emergencies.

B. T.

An Introduction to the Study of the Protozoa, with Special Reference to the Parasitic Forms. By E. A. MINCHIN. Second Impression. Cloth, \$8.50. (London, Edward Arnold, 1922.)

Professor Minchin, whose researches have added many important contributions to the knowledge of zoology, was elected Professor of Protozoology in the University of London in 1906. While there, he wrote this book, which was recognized at once to be a masterful, lucid, and readable presentation of all the main facts about the structure and classification of the protozoa.

Since its publication in 1913, the book has been of great use to all students of systematic protozoology and also to those more directly concerned with the medical aspect of the subject. It attempts to determine the position of the *Protozoa* in nature. To guide the student through the technicalities of the study, all technical terms are defined and explained. While the mutual affinities and interrelationships of these organisms are expressed in as rigid a classification as possible, the author discusses philosophically those parts of the subject where *Protozoa* "throw great light on some of the fundamental mysteries of living matter—as for example, sex—and a special chapter dealing with the physiology of the *Protozoa* has been added."

The author states in his preface that as he is "not a medical man... purely medical problems... as the symptoms and treatment of diseases caused by the *Protozoa*—are not dealt with in this book. The needs of the medical men have, however, been specially kept in view, and the author hopes that the book will succeed in supplying them with useful information, at least from a general zoological or biological standpoint." From this point of view, the reviewer finds that the section of the book dealing with the trypanosomes and allied parasites is the most satisfactory.

In 1915, Professor Minchin died. The volume just issued from the press is an unrevised reprint of the first edition published in 1913. It lacks, therefore, the completeness which it would have had if its author had lived to add to it the knowledge of the protozoa which has been established during the last nine years. In spite of that deficiency, it remains the best book in English on this subject.

S. B. J.

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THE INFLUENCE OF THE TREATMENT OF SYPHILITIC PREGNANT WOMEN UPON THE INCIDENCE OF CONGENITAL SYPHILIS

By J. WHITRIDGE WILLIAMS

In a paper upon *The Value of the Wassermann Reaction in Obstetrics*, which appeared in October 1920, and which was based upon the study of 4547 women whose Wassermann reaction had been determined during the four years ending December 31st, 1919, I considered the significance of the Wassermann reaction during pregnancy. At that time it was shown that in our material syphilis constituted the most important single factor concerned in the causation of fetal death, and was responsible for 34.4 per cent of all deaths occurring between the period of viability and the expiration of the first two weeks of the puerperium. It was, furthermore, shown that the results of treatment were highly satisfactory. When no treatment was instituted 48.5 per cent of the children manifested signs of syphilis, as contrasted with 39.2 per cent and 6.7 per cent, when the treatment was inefficient or efficient, respectively.

Intensive work along these lines has been continued, and has led to a great reduction in the incidence of fetal syphilis in our service. Moreover, in the routine histological examination of the placenta syphilitic lesions are now relatively rarely encountered, instead of very frequently as in the past. In the two years following the completion of the series just referred to, that is, from January 1st, 1920 to December 31st, 1921, 96 of the women included in it have passed through 113 pregnancies in the service, and it has seemed to me that it might be interesting to follow their outcome, especially in regard to the effect of treatment; more particularly as no one has as yet studied a series of syphilitic women who have been under continuous observation for a number of years. Furthermore, the conclusions reached should be especially valuable, as it has been attempted to follow as far as possible the history of all the children concerned. In April and May

of the current year every child that could be traced was visited, so that we have notes on the condition of a large number of the children 4 to 28 months after their birth.

It might be added that in both series the Wassermann tests were made and the treatment instituted in the Syphilitic Department of this Hospital. In talking with Dr. Albert Keidel, concerning the results obtained, he suggested, and I agree with him, that in the former article the differentiation between efficient and inefficient treatment was based upon somewhat uncertain premises and possibly led to erroneous conclusions. In order to insure greater accuracy I have, at his suggestion, classified the treatment under one of the five following groups: A. The administration of at least six doses of diarsenol, followed by mercurials and iodides, and repetition of the treatment until the Wassermann became negative and remained so for at least one year after the cessation of treatment. B. Patients in whom the Wassermann remained persistently negative for six months after the last treatment. C. Patients in whom it did the same for three months after the last treatment. D. Patients in whom a negative Wassermann was attained only shortly before the birth of the child; while Group E includes all patients in whom the treatment was notoriously insufficient, or was not followed by the conversion of a positive into a negative Wassermann, no matter how long it was continued.

Upon analyzing the 113 pregnancies occurring in the 96 women during the two years under consideration, it was found that 9 had ended in abortion and 6 in premature labor. In the former the children were not viable, while in the latter they were either born dead or died during the puerperium, thus leaving 98 pregnancies for consideration. Such an incidence seems high, and at first glance one might be tempted to regard it as a result of syphilis, were it not for the fact that current clinical statistics teach that in normal women at least every fifth pregnancy ends in abortion, which is higher than the incidence here noted. Moreover, upon studying the abortions more critically, it was found that one was induced on account of severe chronic nephritis in the mother. Three occurred in the early months before the effects of syphilis could become manifest, while five occurred between the fifth and sixth month. In three of the latter, autopsy showed no signs of syphilis, and the placental and X-ray findings were likewise negative; while in the other two, autopsies were not obtained but the placenta were normal on histological examination. In four of the six premature labors, the autopsy and placental findings were negative; while in the other two an autopsy was not obtained but the placental findings and maternal Wassermann were negative. Accordingly, while it cannot be categorically stated that syphilis played no part in the production of any of the fifteen abortions or

premature labors, it nevertheless seems permissible to assume that it did not.

Of the 98 children remaining, four died from accidents of labor—one premature separation of the placenta, one prolapse of the umbilical cord and two craniotomies. In each instance, autopsy showed no sign of syphilis. On the other hand, four other children were born dead or died during the puerperium and showed signs of congenital syphilis, thus leaving 90 living children which were discharged from the clinic in apparently good condition.

During the months of April and May (1922) strenuous efforts were made to locate these children, and owing to the persistence of Dr. John G. Murray and our prenatal nurses all but nine of them were found, and were subjected to careful physical examination and a specimen of blood was withdrawn for a Wassermann. This must be regarded as a surprising achievement when the character of the clientele of the service is taken into consideration, and it is recalled that many of the patients had moved several times since leaving the clinic.

Of the 81 children which could be traced, 71 were found to be living and well, manifested no signs of syphilis, presented a negative Wassermann, and were apparently in excellent condition. In another instance, the child could not be traced after the end of the third month, but as it presented a positive Wassermann at that time, as well as at birth, it must be regarded as syphilitic. Of the remaining 9 children, five were living and apparently well, but their mothers would not allow blood to be withdrawn for a Wassermann; while four others had died—three from pneumonia at periods varying from 7 to 14 months after birth and one from gastroenteritis at the age of 11½ months. From these figures, it is apparent that of the 94 children who were born alive, 5 or 5.3 per cent showed demonstrable evidence of syphilis, while 76 were living and well, 9 had been lost track of, and four had died.

The results obtained are graphically shown in Table I which represents an analysis of the 113 pregnancies. In the first five lines in the second column the type of treatment employed is indicated, while in the last two the notation "no treatment" or "spontaneous cure" is made. In the former it may be stated that 13 pregnancies occurred in 12 women, and a word of explanation is necessary concerning the lack of treatment. Ordinarily, it was due to the fact that the patients had registered so late in pregnancy that labor occurred before treatment could be instituted, and sometimes even before the result of the Wassermann test became known; while in other instances the patients had neglected or refused treatment. The eleven pregnancies under the caption of spontaneous cure occurred in nine women, who were not treated for the reason that the Wassermann was already negative, and the history indicated that the disease had disappeared. In these women, after the birth of a varying number of syphilitic

children, the Wassermann had become negative without treatment and subsequently all children were normal. In one patient in the series, this had occurred as early as 1912, while in others the negative Wassermann dated from 1915, 1916, 1917 and 1918 respectively. Such spontaneous cures substantiate the observations of the older writers and indicate how cautious one must be in attempting to evaluate the results of treatment in a given case.

TABLE I.
Analysis of Treatment in 113 Pregnancies.

No. of Cases.	Type of Treatment.	Child L. & W. Wass. negative.	Child L. & W. Wass. positive.	Born alive, Wass. + later.	Born alive, Lost track of.	Congenital syphilis.	Died after discharge.	Born dead, Cause of syphilis.	Premature stillborn.	Abortions.
16	A	10	—	—	1	—	(74)	—	—	3
13	B	10	—	—	1	—	(78)	1	—	1
11	C	9	—	—	—	—	(64)	(64)	(17)	1
20	D	13	3	—	—	1	(50)	(40)	(15)	1
							(100)	(109)	(88)	
29	E	19	—	2	—	2	(44)	(103)	2	2
13	No treat.	2	2	1	2	1	—	—	1	2
11	Spont. cure	8	—	—	3	—	—	—	—	—
113	TOTAL	71	5	1	9	4	4	4	4	6

(The figures in parentheses refer to the serial numbers of the cases.)

The results obtained by the varying types of treatment are graphically shown in another way in Table II. Here it will be noted that the 49 women, who had received type A to D treatment, had gone through 257 pregnancies in the two series. Of these, 172 occurred before and 85 after the institution of treatment, and resulted in the birth of 40.7 and 89.4 per cent of live children, respectively. In this connection it should be noted that we have classified abortions as well as premature labors as dead children. Before the patients came into our hands we had no means of determining the cause of foetal death; so, if similar allowances are made in each group, it seemed that gross figures would give a more correct idea of the results before and after treatment. It should also be noted that a number of these women were treated only in the previous series and received no treatment subsequent to 1920, others were treated in both series, while still others were treated only after January 1920. In either event the results are striking, and indicate that the incidence of dead children and abortions was six times less after than before treatment.

Moreover, comparison of the results recorded in Tables I and II indicates that type A to C treatment gave almost

ideal results, while in Group D they were surprisingly and unexpectedly good. Furthermore, the results noted in Group E were in some respects even more startling, as they show that, from 29 pregnancies occurring in 18 women, 19 normal children were obtained, as demonstrated by examination four to twenty-eight months after birth, notwithstanding the fact that the treatment had been notoriously inefficient when judged by the usually accepted standards. Indeed, in several instances it was noted that the administration of only two or three doses of diarsenol had apparently sufficed to produce a negative Wassermann and to result in the birth of live children.

TABLE II.
Results of Treatment—96 Mothers.

Type of Treatment.	Number of Women.	Pregnancies before Treatment.	Deaths before Treatment.	Pregnancies after Treatment.	Deaths after Treatment.
A	12	47	26	20	3
B	11	39	27	15	0
C	8	28	20	14	2
D	18	58	19	36	4
	49	172	102	85	3
		59.3%		10.6%	
E	26	103	46	48	6
		44.6%		12.5%	
No treat.	12	66	29 + 1 syphilis alive		
		43.9%			
Spont. cure.	9				
	96				

What conclusions can be drawn from the data just adduced? It must at once be admitted that it is difficult to make any precise statement, except that our figures clearly indicate that almost ideal results follow anything like efficient treatment of syphilitic pregnant women, and that surprising results may sometimes follow what would ordinarily be regarded as altogether inefficient treatment in men or in non-pregnant women, which would seem to indicate that pregnant women are unusually amenable to anti-syphilitic treatment. On the other hand, too roseate a view of the possibilities of treatment should not be indulged in; for, while surprising results have been obtained in many patients, others were encountered who were extraordinarily refractory to treatment;—as for example, Case 23, to whom 30 doses of diarsenol were administered without bringing about a negative Wassermann. Notwithstanding this, the child showed no evidence of syphilis and when examined nine months after delivery presented a negative Wassermann.

Such favorable results as we have recorded appear to offer another example of the extent to which syphilis, as it occurs in women during pregnancy, differs from that occurring at other times, as well as in men, and indicates

that there must be something about the pregnant condition which mitigates the virulence of the disease and predisposes to spontaneous cure. Observations tending to support such a proposition were made by Keidel and Moore in studying their patients with neuro-syphilis, when it was noted that extension to the nervous system was less frequent and much more benign in women who had had children than in nulliparous women or in men. Furthermore, all who have had experience in dealing with syphilitic pregnant women, have come to believe that the disease is much more benign in them than in others, and tends in a certain measure to spontaneous cure. Moreover, experiences of this character are in line with the observations of Wade Brown that in experimental animals the pregnant female is very refractory to inoculation with syphilis, while non-pregnant controls are readily infected by the same virus, and tend to indicate that there is something about the pregnant female which seems to interfere with the extension of the spirochete.

At this time it appears futile to discuss the factors which may be concerned in such reactions, but it is apparent that a large field is opening up, which offers unusual opportunities for experimental study to those fitted for it. On the other hand, the practical lesson is that the results already attained afford abundant evidence that the syphilitic pregnant woman is unusually amenable to treatment, and this fact should encourage all who are interested in prenatal work to make every effort to recognize the existence of syphilis and to treat it during pregnancy, as by so doing a normal child may not only be obtained, but the mother can apparently be cured with far greater ease than at any other time.

In this connection a few words may be said concerning the diagnosis of syphilis in pregnant women. From my experience with my own assistants, as well as from what I gather elsewhere, it must be admitted that there exists a general tendency to base the diagnosis almost exclusively upon the presence of a positive Wassermann reaction and

to neglect the clinical study of the patient. In my previous papers I pointed out that the presence of a persistently negative Wassermann on the part of the mother did not preclude the existence of syphilis and the possible birth of syphilitic children, and I have insisted upon the necessity for the greatest care in taking the history and searching for stigmata of the disease. Unfortunately, however, the results obtained in this respect are not very encouraging; as in the 96 women under consideration in this report only 12 presented a history of frank syphilitic infection, while in five others a suggestive history was obtained. Consequently, with the exception of these few cases, we were obliged to base the diagnosis either upon the existence of a positive Wassermann or upon the autopsy findings in the child.

Within the last two years our diagnostic facilities in the case of the newly-born have been greatly enriched by the work of Shipley and Pearson on the use of the X-ray, and we have had abundant opportunity to confirm its value. Naturally, if an autopsy can be obtained and is done by a competent man, the X-ray findings are merely confirmatory, but if an autopsy is not permitted, or the child is still alive, they offer a very important means of diagnosis, as in our experience the characteristic shadow of Wegner's bone disease at the junction between the diaphysis and epiphysis of the long bones must be regarded as pathognomonic.

In *conclusion*, I am aware that the present paper is based upon observations made upon a series of women value, but at the same time the results which we have obtained are highly suggestive and are interesting from two main points of view. First, because our study is based upon observations made upon a series of women with whose past history we have long been familiar, and secondly, because the results obtained are extraordinarily stimulating from the standpoint of treatment and of the great hope which it offers for the future.

THE USE OF LARGE REVERDIN GRAFTS IN THE HEALING OF CHRONIC OSTEOMYELITIS

By MONT R. REID, Cincinnati, Ohio

(From the Surgical Department of the Johns Hopkins Hospital)

The many methods that have been introduced for treating bone cavities following operations for chronic osteomyelitis, attest to the difficulty of dealing with this surgical problem. In general they have been based upon the principle that obliteration of the bone cavity is desirable, and may be grouped under three headings:

(1). *Autoplastic operations with the use of soft tissues.* This idea perhaps originated with Neuber who, in 1886, described a method of treating bone cavities by turning into them flaps of skin and other

soft tissue. This principle has been widely employed.

(2). *Autoplastic operations with the use of bone.* This procedure is essentially the same as the preceding, except that the overhanging edges of bone are used together with the fascia and skin to fill the cavity. (3). *Obliteration by the use of various filling materials.* Schede introduced the method of healing by means of the moist blood-clot; Mosetig-Moorhof used a paste; Senn employed decalcified bone chips, and various surgeons have used fresh detached pieces of fascia, bone, fat and muscle.

The purpose of this paper is to introduce another method of treating certain bone cavities, remaining after operations for chronic osteomyelitis, which the above procedures too often fail to cure. It consists essentially of epithelializing the walls of bone cavities by the use of large Reverdin or pinch grafts. J. P. Lord, in 1902, employed a Thiersch graft in a similar way, but so far as I know, the use of pinch grafts in the treatment of bone cavities has not hitherto been reported. The value of the two methods of grafting will be discussed after the report of two illustrative cases.

CASE 1.—Surgical No. 50382. A white man, aged 37, was admitted December 29, 1919. He complained of swelling, pain and lameness in the left leg.

The infection, beginning acutely in the upper end of the tibia, had been present for twenty years. During all this time, periods of quiescence of the infection with healing of the sinus were followed by periods of pain and swelling of the leg which were relieved by the discharge of pus and sequestra.

Operation, December 31, 1919. The cavity in the upper end of the tibia, measuring two inches in length and half an inch in diameter, was found filled with granulation tissue. This was removed with a curette. An attempt was then made to obliterate the cavity by cutting away its bony walls, but its position in the tuberosity of the tibia and the proximity of the knee-joint made this impossible. The operation terminated, therefore, in an attempt to secure healing by Schede's moist blood-clot method.

After three weeks, during which the healing of the wound seemed quite satisfactory, there developed signs of a mild infection. This subsequently necessitated the opening of the wound and the removal of the infected blood-clot. The cavity was then treated by the Carrel-Dakin method. When it became lined with healthy, relatively sterile granulation tissue it was epithelialized by the use of thick pinch-grafts removed from the thigh.

Since the patient's discharge from the hospital March 21, 1920, there has been no further trouble with his leg (*vide* Fig. 1).

CASE 2.—Surgical No. 49232. A white man, aged 55, was admitted August 6, 1919. He complained of "bone disease in the right leg."

The infection of the bone had begun when the patient was 9 years old, and for forty-six years his leg had many times discharged pus and fragments of bone. Periods of incapacity had varied from several months to a year. During the many years of his affliction the only surgical treatment of the disease had consisted in the incision of superficial abscesses.

FIG. 2 shows the great increase in the size of the right leg, the numerous scars of previously discharging sinuses, and, what has often been noted in long-standing cases of osteomyelitis, the increase in the length of the leg. In this instance the right tibia measured 3 cm. longer than the left.

Operation, August 8, 1919. Numerous small abscesses each containing a tiny sequestrum were present throughout the diseased portion of the tibia. The process was so extensive that the operation resulted in the production of a large boat-shaped cavity which extended from the tuberosities to the malleolus.

From August 8 until September 10 the wound was treated with Dakin's solution. The granulating surface was then covered with thick pinch-grafts which promptly grew and covered the surface of the cavity with epithelium.

February 17, 1920 (*vide* Fig. 3). The cavity was well lined with skin which showed no tendency toward maceration. The photograph, unfortunately, does not show the limits of the upper

end of the cavity which extended far into the tuberosities of the tibia.

Technique of skin grafting.—The bone cavity is treated with Dakin's solution until it becomes lined with clean firm granulations. Without the use of Dakin's or some other antiseptic solution the granulation tissue becomes oedematous or "sea-weedy," and forms an unhealthy base for the growth of grafts. Two hours after the last irrigation with Dakin's solution large thick pinch-grafts, half a centimeter in diameter are placed closely together upon the surface of the cavity. The grafted wound is then exposed to the air for from six to eight hours. This short drying period serves to fix the grafts firmly to the granulation tissue. The grafts are then covered and held in place with a single layer of gauze which is firmly secured to the normal skin so that the moistening and changing of saline compresses during the next two days will not displace the grafts. After two days the grafts have taken and the use of Dakin's solution instead of salt solution is begun. This is applied by laying wet compresses directly against the wound (the thin layer of protecting gauze having been removed) every two hours during the day and every four hours at night. After about five days the Dakin's solution is discontinued and the wound is dressed with rubber "protective" or old linen. Invariably the grafts grow quickly and cover the granulation tissue with epithelium in from ten days to two weeks. Should the granulations become high (usually from the oedema of infection) caustics should not be used; Dakin's solution reapplied for one or two days will reduce the swollen granulations to the level of the grafts, and will not injure the growth of new skin.

Indications for skin-grafting.—The cure of chronic osteomyelitis when the disease is confined to the shaft of a long bone is usually not difficult, for the surgeon can frequently remove bone enough to prevent the formation of a cavity or else to permit soft tissues to fall readily into a shallow cavity. But in the ends of long bones, particularly the upper and lower ends of the tibia and occasionally the lower end of the femur, the successful treatment of a bone cavity is made difficult by the presence of the joint which limits the amount of bone that may be chiseled away. In such locations the surgeon has always to face the problem of dealing with a deep cavity, and it is in such cases that we have for the past four years made use of thick pinch-grafts.

Again, when the shaft of the tibia is greatly enlarged, a thorough removal of all sequestra and foci of infection may often result in a cavity that cannot be obliterated by the removal of bone or by the infolding of the scant soft tissues that cover this bone. One occasionally encounters a similar condition in the humerus. Whenever, therefore, we have a large bone-cavity that is difficult to treat by other methods, the use of pinch-grafts makes it possible

to secure a rapid epithelialization of the wound.* (*vide* Figs. 2, 3.)

We have adopted the use of pinch-grafts for several reasons. The epithelial covering which results is thicker and more durable than that obtained by the use of Thiersch grafts. In deep cavities there is always a tendency toward the formation of moisture which will macerate delicate epithelium, and in the bone cavities of which we have been speaking, we have found that the thick pinch-grafts are tough enough to withstand this tendency to maceration, whereas Thiersch grafts often appear to melt away after taking.†

Another factor in favor of the use of pinch-grafts is the certainty of their taking. On a surface properly prepared with Dakin's solution practically every graft will take. Thiersch grafts, on the other hand, do not grow as constantly, for the impossibility of sterilizing completely a granulating surface will often result in the accumulation of pus which may lift these grafts from their base.

Changes in the size of the grafted bone cavity.—The fate of the epithelialized bone-cavity has been the subject of an interesting study. For several years we have been interested in watching the changes that take place after grafting deep wounds of the soft parts. Ample opportunity for this study has been afforded by the cases of carbuncle which it has been our practice to excise completely and graft within a few days. In this way, we have

* Another application of the method has been useful in a few cases of extensive acute osteomyelitis of the tibia when, at the time of the primary operation, it has seemed necessary to split the periosteum throughout its length. In each instance the periosteum quickly retracted and the wound presented the appearance of extruding the shaft of the tibia. After from four to six weeks the removal of the sequestered tibia left a relatively flat involucrum covered with granulation tissue. These wounds were treated with Dakin's solution and then epithelialized with the aid of large Reverdin grafts. The results have been the substitution of flat bones for the normal tibiae. In the few cases that have been followed these new-formed bones have tended to become round.

† Reverdin's original grafts were very small. He describes them as epidermic grafts. However, he points out that his grafts included the whole epidermis and very little of the dermis. By preparing a more nearly sterile granulating surface with Dakin's solution we can at the present time successfully transplant very large Reverdin grafts. In the center of these large grafts the entire thickness of the skin is included. Though differing from Reverdin's grafts in that they are larger and thicker, the principle remains the same and the present day pinch-grafts should still, I think, be called after Reverdin.

produced many epithelialized depressions of the soft tissues. Nature corrects to a remarkable degree this cosmetic blemish, for after several months the normal contour of the body is re-established to a surprising degree. This subject will be dealt with more fully in a paper on wound healing and wound contraction. It is mentioned here because of a similar process which takes place in epithelialized bone cavities. The patient upon whom Lord operated wrote after returning home that the cavity in his bone was much smaller. Ignorant of this observation, Dr. W. F. Rienhoff and I began studying the changes in the size and shape of the bone cavities in our cases. This has been done by making plaster casts of the cavities before grafting and at frequent intervals afterward. In Fig. 5 is shown a photograph of a series of casts from one patient. The leveling of the sides and the decrease in the depth of these cavities form a striking example of nature's attempt to obliterate a cosmetic defect. Whether or not epithelialized bone cavities may be completely obliterated has not yet been determined.

With such a method of treating chronic osteomyelitic bone cavities the time required for curing the most stubborn cases is usually from four to six weeks. This means a great economic saving. Considering the chronicity of this disease and the difficulty of curing it, the resultant deformity is of no moment to the patient. In a recent case in which the patient had suffered for thirty years and the ankle joint had become completely ankylosed, I grafted a large cavity running entirely through the ankle (*vide* Fig. 6).

Everyone is aware of the liability to recurrence of the infection after any of the other methods of treating chronic osteomyelitis. In the method I am proposing there should be but a relatively slight chance for recurrence; but that it may not recur must for the time being remain an undetermined point.

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FIG. 1.—Case 1. Surgical No. 50382.

Johns Hopkins Hospital.

On the thigh are the scars that resulted from cutting the pinch grafts. In the bone cavity the grafts are still visible. Photograph taken fourteen months after the patient's discharge from the Hospital.



FIG. 2.—Case 2. Surgical No. 49232.

Johns Hopkins Hospital.

Duration of the disease 46 years. Note the increase in size and length of the right tibia.

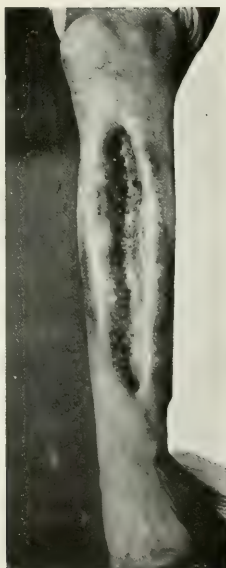


FIG. 3.—Case 2.

Several months after epithelialization of the bone cavity. The extent of the cavity into the ends of the tibia is not shown in the photograph. The grafts can be seen.



FIG. 4.—Surgical No. G2812. Cincinnati General Hospital.

Bone cavity is shown completely epithelialized two weeks after being grafted with large pinch grafts.

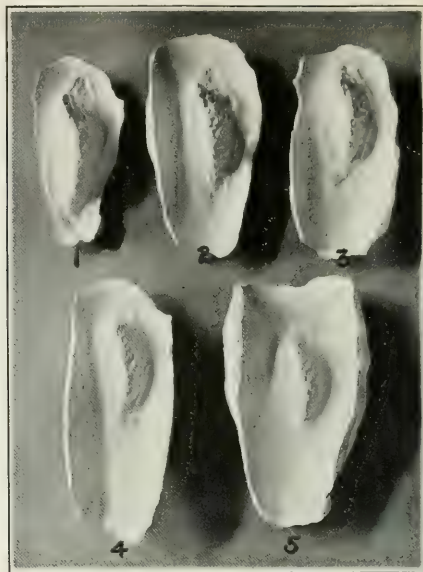


FIG. 5. Surgical No. 53038. Johns Hopkins Hospital.

Plaster molds of an epithelialized bone cavity: 1 day before, 5 days after, 18 days after, 42 days after and 10 months after skin grafting. The decrease in the size of the cavity is plainly shown in the molds.



FIG. 6.—Surgical No. 56249 Johns Hopkins Hospital.

The joint was ankylosed by a chronic osteomyelitis of thirty years duration. The arrow shows an epithelialized cavity extending through the destroyed joint.



FIG. 7.—Surgical No. 55878. Johns Hopkins Hospital.

Bone cavity three weeks after grafting it with large pinch grafts.

THE MORPHOLOGICAL THEORY OF MONOCHORIONIC TWINS AS ILLUSTRATED BY A SERIES OF SUPPOSED EARLY TWIN EMBRYOS OF THE PIG

By GEORGE W. CORNER

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The subject of identical twins is of such interest from many biological viewpoints that conjecture as to the mechanism of their origin has far outrun our actual information. Almost the whole mass of discussion now current in the literature of embryology and genetics is pure hypothesis constructed by reasoning backward from the observed anatomy of late stages, chiefly from the disposition of the foetal membranes at birth in cases of twinning. Even the term "single-ovum twins" is an assumption, when applied to mammals.

So far as is known to the present writer the extant specimens of monochorionic twin embryos, in stages early enough to be of value for morphogenetic study, are (1) the blastodermic vesicle of the sheep, about 7 days old, and a similar embryo of the ferret (*Putorius*), described by Assheton ('98), in each of which two inner cell masses are found in one blastocyst; (2) the human embryo "Mateer" described by Streeter ('19a), in which a similar arrangement is supposed to exist at a slightly later stage of development; (3) two human specimens, each containing twin embryos about 12 mm. long, recently described by Arey ('22); and (4) the complete series of embryos of the nine-banded armadillo (a species in which single-ovum quadruplets are produced at every birth) which we owe to the work of Newman and Patterson ('10, '13). With Assheton's specimens and his own as the basis for hypothesis, Streeter ('19b) has constructed a very plausible series of diagrammatic stages of human single-ovum twin-formation, in which this phenomenon is represented as due to the production of two embryonic areas of inner cell masses on the inside of a single blastocyst, before the formation of the amnion. Such a hypothesis fits in well with the usual disposition of the foetal membranes at birth; and it would also readily account for the appearance of monochorionic twinning in any mammalian species, since all mammalian embryos, whatever their later divergencies of form, pass through an essentially similar blastocyst stage. Such a view of twinning would furthermore accord with the fact of general occurrence of polyembryony in the animal kingdom, and would give added philosophical value to the experimental production of uniovular twins and double monsters in fishes and amphibians.

A different view has been proposed by Newman in his interesting book "The Biology of Twins" ('17), upon the

basis of his studies of the armadillo. In this species development proceeds as in those mammals which undergo so-called "inversion of the germ-layers," until the amnion is formed; then the embryonic area divides into four embryos, and each embryo migrates down a meridian of the chorion, dragging with it the surrounding portion of the amnion, until finally a single chorion contains four embryos, each enclosed in a portion of the original amnion, now drawn out into four sacs with a common central connection. It is obvious that this form of polyembryony cannot take place except in mammals in which the formation of the embryo proper is delayed until after the appearance of the amniotic cavity; and since in man a similar progenesis of the amnion occurs, Newman has been led to believe that in man also monochorionic twin-formation follows the same scheme as the polyembryony of the armadillo. Furthermore, if in mammals monochorionic twinning is indeed dependent upon progenesis of the amnion, then it cannot occur in species in which the amnion is produced by rolling up of the chorion over the embryo. Newman has been willing to make this assumption, and in his book has ventured the prediction that single-ovum twins will probably never be found in ungulates.

The three specimens of early embryos of the pig which are to be described in the following pages all came into the author's hands within the space of a few weeks, at the packing house of Joseph Stern and Company, in New York City.* It is remarkable that all three were found among about 500 pregnant swine examined in this one abattoir, whereas similar specimens have never been seen in many hundreds of sows at other places. The packing-house in Baltimore which has furnished pig embryos to the Johns Hopkins laboratories for thirty years has never yielded, as far as the writer knows, anything resembling monochorionic twins except one double monster and one specimen to be mentioned later, in which it was not certain whether one or two chorions were present.

The following descriptions and illustrations will be clear to the reader who is not familiar with the specific embryology of the pig, if he will recall that the blastocyst

* The writer was at the time working under the auspices of the Department of Experimental Evolution of the Carnegie Institution of Washington, at Cold Spring Harbor, Long Island. To the Director, Dr. C. B. Davenport, thanks are due for providing support and assistance.

does not remain spherical, but is rapidly elongated to 30 or more centimeters, so that by the fifteenth day the embryo itself is a small thickening upon a very narrow but immensely elongated cylindrical chorion. The chorion does not develop villi, as does the human gestation sac, but remains smooth, and can at all times be readily peeled out of the uterus. The allantois reaches such size as to fill a large part of the cylindrical chorionic cavity, and ultimately becomes adherent to the inside of the chorion, giving rise to a diffuse allantoic placentation.

SPECIMEN 1.—The uterus of sow C-155 contained 14 single embryos and the twins to be described. Eight of the single embryos were normal at the stage of 33-34 somites (*i. e.* of the 3rd week). Six showed abnormalities as follows: Two were retarded, without gross defects; one was retarded, with extreme flexure; one was slightly retarded, with undistended allantois. In another the embryo itself appeared normal, but its allantois was entirely cut off from the embryo by the amnion; the embryonic stump of the allantois was closed over, and the extra-amniotic part of the allantois which was adherent to the chorion had upon it a small highly vascularized vesicle. Finally, one embryo was represented merely by an almost amorphous chorionic mass. Since there were 16 corpora lutea, it seems that one of the embryos was totally missing.

In the twin specimen (Fig. 1), two embryos lay about 18 mm. apart within one continuous chorion of dimensions quite similar to those of the normal embryos in the same uterus. Fluid injected into the chorionic cavity dilated the whole space; that is, there was no septum between the embryos. When the cylindrical chorion was slit open, it was found that both embryos were abnormal as illustrated. In one, the allantois was entirely cut off from the embryo, and was growing in an almost unexpanded condition upon the chorion adjacent to the embryo. In the other, the allantois had been converted into a cluster of small irregularly dilated vesicles. The yolk-sac vessels of this embryo had not grown, as normally, out along the yolk-stalk onto the sac, but remained as a convoluted nodule on the yolk-sac near the umbilicus. That portion of the yolk-sac (or sacs) lying between the embryos had become curiously twisted and vesiculated, as shown in Fig. 1, so that it was unfortunately impossible to determine whether the two embryos had originally possessed a common yolk-sac. The two amnions seemed perfectly normal; the enclosed embryos were slightly smaller than the normal single embryos in the same uterus.

SPECIMEN 2.—The uterus of sow C-392 contained 10 normal embryos of 11 mm. crown-rump length, and one pair of twin embryos enclosed in a single chorion (Fig. 2). There were 14 corpora lutea; three ova were therefore missing, a proportion not much above the average loss of ova in this species. The twin embryos appeared quite normal, but one of them occupied, with its allantois, about two-thirds of the available chorionic cavity, leaving but one-third for the other. The embryo with the larger allantois was 9.5 mm. crown-rump length, the embryo with the smaller allantois was 8.5 mm.; there was no significant difference in the development as indicated by external form. Injection of fluid into the allantoic cavities showed that the two were not continuous. When the chorion was opened, it appeared that the yolk-sacs, which at this stage in the development of the pig are beginning to be obliterated by pressure of the allantois, were in the twin specimen very much twisted. It was thus again impossible to determine the original relation of the yolk-sacs, for although they were entirely separate in this specimen, the separation might conceivably have been produced by torsion, as the figure clearly shows.

SPECIMEN 3.—The uterus of sow C-66 contained 7 normal embryos of 23 mm. crown-rump length and a pair of twin embryos. There were 15 corpora lutea. Fig. 3 shows that the arrangement was somewhat different from that in the other two specimens, for here the two embryos were not placed end to end in the chorionic cavity, but side by side. The allantoides were expanded so fully that they came entirely into contact with the inner chorionic surface, obliterating the chorionic cavity. Each amnion was now surrounded by its allantois, as usual at this stage, so that each embryo with its amnion appeared to occupy a separate cavity divided from the other by a septum. The cavity was obviously the allantoic cavity and the septum was formed by the apposed walls of the two allantoides. The areas of attachment to the chorionic wall were so near together that the two umbilical cords appeared to spring from almost the same point, in the line of reflection of the allantoic septum. A section taken through this line showed clearly (Fig. 4) that the chorion was continuous over the surface, not dipping between the allantoic walls; that is, there was a single chorion containing two allantoides. Obliteration of the yolk-sacs had proceeded so far that their relation to each other could not be made out; they seemed to be separate. The embryos occupied approximately equal parts of the chorionic cavity. One was 16 mm. crown-rump length and the other 15 mm. These embryos were sufficiently advanced to permit determination of their sex by the criterion described by Spaulding ('21), which (as Dr. Spaulding assures the author) can be applied to the pig as well as to human embryos. In both these twin embryos the urethral groove runs on to the glans portion of the phallus, and they are both therefore presumably of the male sex.

DISCUSSION

The specimens which have been described and illustrated may obviously be explained by the assumption that each is an example of twins arising within a single blastocyst, or by the alternative supposition that a deceptive appearance of twinning arose by the fusion, in each case, of two originally separate blastocysts. This latter possibility seems unlikely; one would expect to find, in addition to specimens like these, other and more numerous examples not so deceptively perfect. Such fusion is at least not common in early stages of development of the pig; the present writer has never seen it during the study of hundreds of embryos of the first three weeks. It is true that after mid-pregnancy the adjacent necrotic ends of two chorionic sacs occasionally adhere or invaginate in a more or less complete way, but in such cases the larger total dimension of the combined chorions, the considerable distance between the fetuses, and the presence of a chorionic necrosis or invagination at the region of fusion gives sufficient evidence of the dual origin of the fetus. In the two earlier specimens under consideration there was no evidence, to the eye or under the dissecting microscope, of any chorionic septum between the embryos, and in the third specimen there was microscopic evidence that the chorionic space was single, *i. e.*, that the septum was formed by the internal membranes alone, without participation of the chorion. On the whole, the theory that the specimens were monochorionic twins seems more

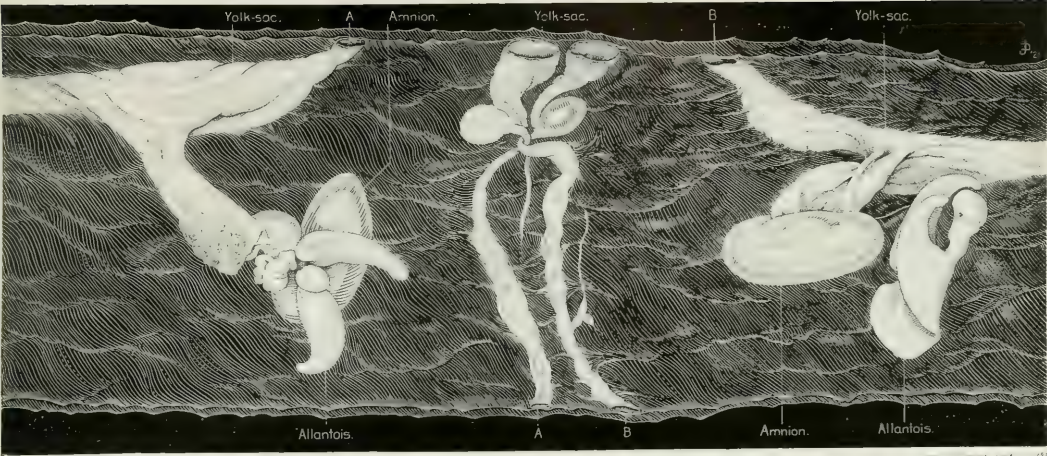


FIG. 1.

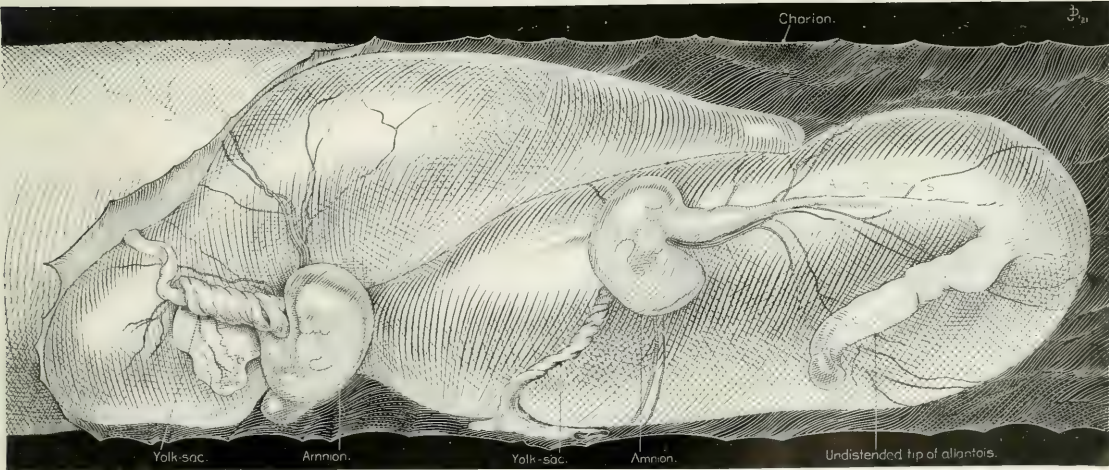


FIG. 2.

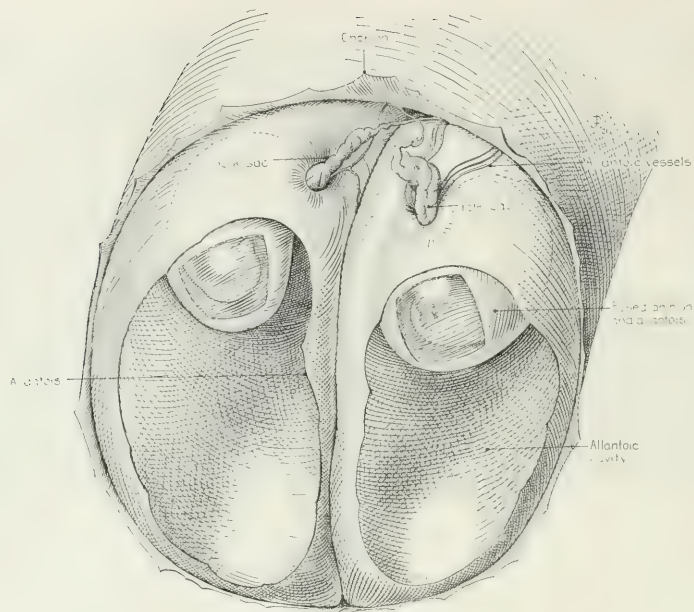


FIG. 3.

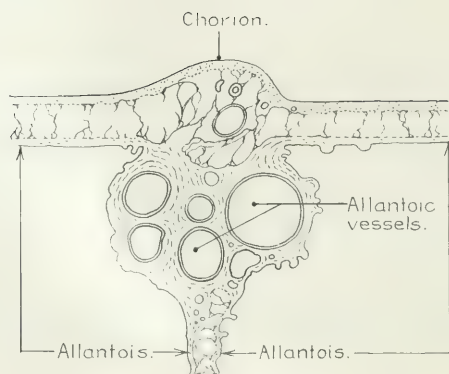


FIG. 4.

FIG. 5.

Explanation of Pig Twinning.

Single

Twin

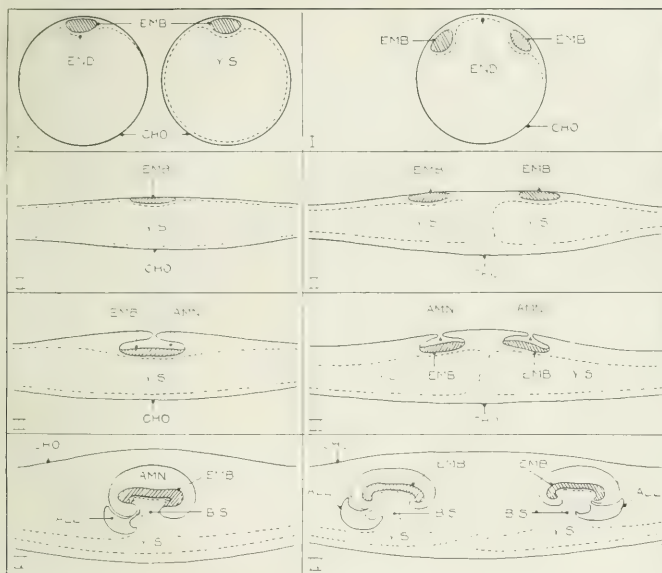
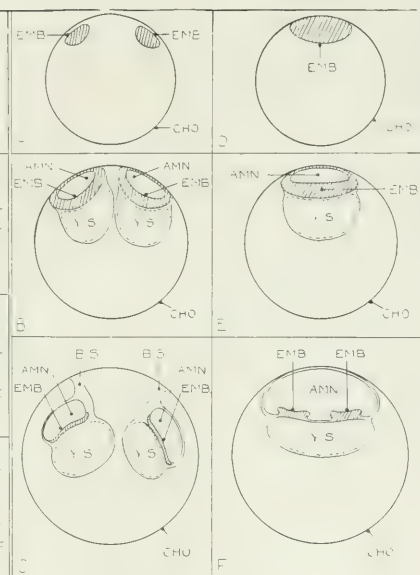


FIG. 6.

Hypothetical Diagrams of Human Monochorionic Twins.

Duplication before
formation of
amniotic cavityDuplication after
formation of
amniotic cavity

probable than that they were examples of accidental fusion of separate blastocysts with obliteration of the intervening trophoblastic (chorionic) wall. It should be mentioned at this point that in a specimen from a local packing-house, studied through the courtesy of Dr. G. L. Streeter, which appeared at first sight to be another case of mono chorionic twinning, in mid-pregnancy (fetuses 130 mm. long), dissection revealed evidences hinting at a possible origin from two separate chorions, through the extensive invagination or penetration of one by the other. The impression gained from all of these specimens taken together is in accord with the recent views of Arey (1922) formulated after study of twin tubal pregnancies as recorded in the Carnegie Embryological Collection, that the appearance of mono chorionic twinning may, at times be due to the fusion of two chorions, but that true polyembryony may also occur.

Assuming that our specimens are indeed mono chorionic twins, it is possible to reconstruct the stages of their formation by means of a series of diagrams involving but one or two hypothetical steps. In Fig. 5, I to IV, such a series is placed in parallel with the actually known stages of development of the single pig embryo. Stage I in the twin series is borrowed from the sheep of Assheton's description, stages II and III are hypothetical, and stage IV is specimen 1 of the author's collection. In this scheme it is assumed that twinning is first made evident by the appearance of two embryonic areas (inner cell-masses) on the inner surface of a single blastocyst. Following this stage we may suppose that an amnion is formed about each embryonic area exactly as in single embryos. Meanwhile the endoderm has been spreading around the inside of the blastocyst. At the same time the blastodermic vesicle has been undergoing the great elongation which is characteristic of the pig and allied ungulate species. Whether the endoderm would usually form a single inner lining of the blastocyst, and thus provide both embryos with a single common yolk-sac, or pinch off into two yolk-sacs, is a matter for conjecture, but in preparing the accompanying diagrams the author has preferred to follow his actual specimens and to represent the yolk-sacs as doubled structures. The result, as shown in Fig. 5, stage IV, (also, Fig. 1, specimen 1) would be a single chorionic vesicle bearing separate embryos, each enclosed in its own amnion, with a yolk-sac which, for all we know at present, might be either doubled or common to both embryos. Each embryo would thereafter produce its own allantois, and when the allantoides had expanded to a moderate degree we would have the stage represented by Fig. 2 (specimen 2). Next the allantoides would expand so voluminously as to surround the amniotic membranes completely, as in Fig. 3 (specimen 3), in which we have a single chorion containing two allantoic cavities with the amnions invaginating the allantoic walls and the yolk-sacs passing out through the

necks of the invaginations to be between the allantoic walls and the chorion. Finally, as the expansion of the amniotic cavities brought about complete fusion of the three fetal membranes, the chorionic cavity would be lined at all points by both allantoic and amniotic tissue, and would be divided into two cavities by the abutting portions of the respective allantoic-amniotic membranes.

It thus seems apparent that mono chorionic twinning in ungulates is not to be considered impossible merely because in mammals of this order the embryonic area is well differentiated before the amniotic cavity arises. Progenesis of the amnion may no doubt influence the morphology of twins, but the occasional occurrence of mono chorionic twins is not thereby limited to any special kinds of mammals.

In conclusion, we may permit ourselves to leave the more or less solid ground afforded by our three specimens, and to indulge in a brief speculation regarding the morphogenesis of human mono chorionic twins as suggested by our studies of polyembryony in the pig. We have shown the probability that, in the pig, twinning must already be apparent before the formation of the amnion, while in the armadillo, as investigated by Newman and Paterson, the polyembryonic budding of the four embryos awaits the formation of an amniotic cavity, which is thus common to all the embryos of one ovum. It may also be pointed out that the human embryo, like all other mammalia, must first pass through a stage of simple blastocyst structure with an inner cell mass, before it proceeds to the splitting open of the amniotic cavity. It is true, as Newman points out, that at this latter stage the morphological relations are much like those at which in the armadillo multiplication of the embryonic areas begins; but it is also true that at the earlier blastocyst stage there is opportunity for twinning as in the pig, by the appearance of two inner cell masses instead of one. Our hypothesis, illustrated diagrammatically in Fig. 6, is that human single-ovum twins may be of two types. One sort of twinning, (Fig. 6, A. B. C.) arising by duplication of the inner cell mass before formation of the amniotic cavity (pig type) would typically give rise to two embryos in a single chorion with two independent amniotic cavities. The "Mateer" embryo as interpreted by Streeter illustrates this relation, and it is this scheme of twin-formation which is propounded in Streeter's contribution of 1919. A second kind of twinning, occurring by duplication of the actual embryonic areas after formation of the amniotic cavity (armadillo type), would produce two embryos within a single amnion (Fig. 6, D. E. F.). There would probably be intermediate and unclassifiable stages, because the moment of separation of the embryos might be intermediate in time, (*i. e.*, during the opening of the amniotic cavity) and also because in the first type the embryos might lie so near together that the separate amniotic cavities would fuse. Our theory thus

covers, perhaps too easily, the known variations of arrangement of the fetal membranes of twins in pre-natal stages and at birth. (For collections of such data see O. Schulze, 1897, and Wilder, 1904). The conception that there may be a series of types of human single-ovum twins accords well with the fact that there is a rather wide variability in the degree of resemblance between so-called "identical twins;" but caution suggests that we leave these concluding notions to await the further test of actual specimens.

LEGENDS OF FIGURES

FIG. 1.—Specimen 1. Embryos of the third week, showing the cylindrical chorion cut open to expose the embryos. Points indicated by the letters A-A were adjacent before the yolk-sac was cut in opening the chorion, as were points B-B. Note abnormalities of the allantoides and yolk-sacs as described in the text. ($\times 7$.)

FIG. 2.—Specimen 2. Normal embryos 8.5 and 9.5 mm. long, enclosed in a single chorion. ($\times 2.5$.)

FIG. 3.—Specimen 3. Semi-diagrammatic. The embryos lie side by side within a single chorion. In this stage the amnions have invaginated the allantoides. The yolk-sacs appear to be separate, as in the previous specimens. ($\times 2.5$.)

FIG. 4.—Diagram of a section taken through the chorion and allantoic septum near the point of attachment of the embryos of Specimen 3, showing that the chorion does not participate in the formation of the septum.

FIG. 5.—Explanation of Pig Twinning.
Single Twin

Fig. 6.—Hypothetical Diagrams of Human Monochorionic Twins.
Duplication before Duplication after
formation of formation of
amniotic cavity amniotic cavity

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DEVELOPMENT AND GROWTH OF THE METANEPHROS OR PERMANENT KIDNEY IN CHICK EMBRYOS

(EIGHT TO TEN DAYS' INCUBATION)

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The story of the development of the metanephros, the organ which forms the permanent kidney in the higher mammals, occupies a voluminous literature and is based on the work of many competent observers extending over a long period of time. Herring (1900), Schreiner (1902), Stoerk (1904), Huber (1905), and Felix (1914), in their classical contributions to the subject, have given a very complete résumé of the work of previous observers whose investigations, made by means of fixed sections and reconstructions, were continued until this method could yield no further results. However, with the perfection of the tissue-culture method it became evident that by this means of approach new results might be obtained in this field. Carrel and Burrows (1910) were the first to make cultures of the kidney. They observed, after five or six days, that tubes had grown into the plasma for a short distance and that there seemed to be a lumen limited by

epithelial-like cells. These tubes had, they thought, the appearance of renal tubules. Champy, in 1914, also cultivated renal tissue, but he claimed that a dedifferentiation of the entire renal anlage into an indifferent epithelium took place. In 1920 Atterbury grafted the metanephric anlage of chick embryos (6 to 7 days' incubation) in the allantois, with the result that the already formed epithelial tubules proliferated and the less differentiated nephrogenic tissue acquired an epithelial arrangement. Hematopoiesis also occurred and glomeruli developed.

In the hope of clearing up some of the controversial points in the development of the kidney, a study of the metanephros by means of cultures, as well as by fixed serial sections and spreads of living tissue, was undertaken. The results obtained in regard to the earliest stages of development of the metanephros, the anlage and early developmental stages of the renal evagination and

nephrogenic tissue, and the later development of the uriniferous tubules, confirmed those of Schreiner and Huber and therefore need no further discussion. Only the observations that differ materially from those of previous observers, such as the growth of the collecting tubules, differentiation of the anlage of the uriniferous tubules, and development of the glomerulus, together with the formation of the blood-vascular system, will be discussed in this paper.

I am indebted to Dr. and Mrs. Warren H. Lewis, not only for advice and assistance, but also for the technique employed, which made possible the cultivation and observation of the renal tissue.

MATERIAL AND METHODS

Cultures were made by explanting small pieces of chick embryos after from 8 to 10 days' incubation. The pieces to be explanted were chosen with the aid of the dissecting microscope from the most inferior medial pole of the mesonephros and the superior medial pole of the metanephros, because, as is commonly known, the differentiation of the mesonephros proceeds antero-posteriorly, whereas in the metanephros it is postero-anteriorly. For this reason the degree of differentiation at any age depends to some extent on the part of the renal body from which the specimen is taken. The areas chosen were the least differentiated embryologically and the most isolated anatomically. The tissue was cut up into pieces as thin as possible and placed on sterile coverslips, in a small drop of Locke-Lewis solution (85 c.c. Locke's solution plus 15 c.c. chicken bouillon plus 0.5% dextrose). The coverslip was then inverted so as to form a hanging drop, sealed onto a ring of vaseline, and incubated at 39° C. The cultures were observed at varying intervals in a warm box at 39° C. Some of them, after varying periods of growth, were fixed either by means of iodine vapor or of Zenker's fluid and stained. Serial paraffin sections 5 micra in thickness were cut, stained with hematoxylin-eosin, and mounted separately. The entire metanephric body of both injected and non-injected chicks was studied at different ages in fresh spreads.

MORPHOLOGY

The urino-genital system of chick embryos from 6 to 10 days' incubation can be dissected with considerable ease without the microscope. When the abdominal cavity is opened and the stomach and intestines have been removed, the mesonephric bodies appear as two greenish-red structures filling up a large part of the remaining space in the abdomen. They are situated one on each side of the vertebral column and extend from just below the attachment of the liver to a point immediately above the bifurcation of the abdominal aorta. They are more or less bilaterally symmetrical and seem to be connected across the mid-line

by only a thin sheet of mesothelium. Their dimensions vary according to the age of the embryo; between 6 and 10 days' incubation they average about 1 mm. in diameter and about 3 mm. in length. They are somewhat wedge-shaped pyramidal bodies, diminishing in width toward the caudal pole. The color is characteristic and is in marked contrast to that of the metanephroi and other abdominal viscera. Over the glistening surface can be seen many pin-point red spots, the glomeruli. At the upper (cephalic) poles are the gonads, two opaque white tubular-like bodies, which, starting above and behind the pointed upper poles of the mesonephric bodies, continue across the uppermost anterior surfaces and course caudward along the antero-medial margins, ending in a point corresponding to one-half the length of the mesonephric bodies. From the dorsal surface, and continuing below the lower pole of each mesonephric body, is the mesonephric or Wolffian duct, which serves as the excretory duct for the mesonephric body or embryonic kidney. These small white ducts approximate each other in a downward course and end in the cloaca. The branching connections of the Wolffian duct with the mesonephros, the mesonephric ureters, could be distinctly seen by elevating the lateral margins of the mesonephric bodies. Along the vertebral column, dorsal and also immediately posterior to the mesonephric bodies, lie two bilaterally symmetrical translucent structures, the metanephroi. The genito-urinary apparatus is entirely covered by a thin layer of cubical epithelium which is known as mesothelium or coelomic epithelium. In the youngest ages, the metanephros is completely obscured by the overlying mesonephros, which reaches its greatest volume at about the eighth day. From this time on, however, the metanephros increases rapidly in size, with the result that in a 10-day embryo the mesonephros appears to be a relatively small body situated on the anterior surface of the metanephros. The supply of blood to the metanephric body between these ages is very small, while the blood-flow through the mesonephros is quite abundant, a fact which accounts for the difference in the color of the two bodies. The shape of the metanephros changes constantly during the growth and development of the embryo, so that no definite shape can be described as typical for all ages. However, in general it may be said that the shape approximates that of a dumb-bell, being broad at both poles and narrow in the middle. This is well shown for older embryos (18 days) by Minoura (1921, Fig. 8). At the age of 6 days the metanephric body has a smooth exterior surface, with no lobulations. A little later, however, the bodies begin to show definite lobulations, which by the tenth day have become discrete lobes completely covered by coelomic epithelium.

The entire metanephros was dissected out and placed on a slide, thus making it possible to study the fresh tissue at different ages with the aid of the microscope.

In the 6-day embryo it was found that the metanephric tubule, which sprouts off the Wolffian duct, had already grown into the metanephric body, completely traversing the undifferentiated tissue from the posterior to the anterior pole. Along the entire length of the metanephric tubule the primary collecting tubules had budded off in three main planes, lateral, dorsal, and ventral, and there seemed to be an enlargement of the lumen of the metanephric tubule at the anterior and posterior poles, at which places the metanephric body is broader. Each of the primary collecting tubules, although they had grown only a slight distance from the metanephric tubule, had just divided dichotomously into two secondary collecting tubules. No further division, that is, beyond the secondary tubules, was ever observed at this age. Immediately about the primary and secondary collecting tubules was a sharply defined covering, consisting of a single layer of endothelial cells. This structure will be described in detail later. Between the bases of the primary collecting tubules, near the posterior pole of the metanephric body, sinuses had differentiated out of the tissue surrounding the bases of these primary collecting tubules. These spaces or sinuses were lined with flat endothelial cells which gave rise to blood elements. This was the first appearance of sinus formation observed in the metanephrogenic body.

At 7 days the branches of the primary collecting tubules, namely, the secondary tubules, had again divided dichotomously into tertiary tubules. The branching appeared always to occur in a forked manner, the tubules growing toward the periphery. There was a marked increase in the length of the primary and secondary collecting tubules during the sprouting of the tertiary tubules. The apparent dilatation of the lumen of the metanephric tubule at the anterior and posterior poles had disappeared by this time, but in these areas there was a much more rapid growth of the collecting tubules than in the intermediate region. This growth was perhaps somewhat more pronounced at the posterior than at the anterior pole, for, as is well known, the metanephros differentiates postero-anteriorly. In the body of the metanephros the formation of sinuses proceeded hand-in-hand with the elaboration of the collecting-tubule tree, replacing the undifferentiated tissue about the bases of the primary and secondary collecting tubules. Towards the periphery of the metanephric body, strands of mesenchymal cells, arranged in festoons, divided the cortex into a series of lobes which corresponded roughly to the secondary collecting tubules. The definition of this lobulation was more pronounced in the 8-day embryo, constituting complete separation of the metanephrogenic lobules into distinct lobes surrounded entirely by a layer of cubical cells and arranged always in relation to the secondary collecting tubules. The length of the primary, secondary, and tertiary tubules increased greatly with

this lobulation, the tertiary tubules being entirely intralobar, while the primary, as well as the secondary tubules, were extralobar. In the anterior and posterior poles of the metanephros the tubules grew to a greater length and gave off more sprouts, resulting in a greater number of secondary and tertiary branches in these regions. As has been stated above, the lobes were established in the 8-day embryo, but an intralobar lobulation was observed to begin immediately following the lobe formation. This subdivision of the lobe into smaller lobules persisted until the elaboration of the collecting tubule tree was complete. In the 9-day embryo it corresponded to the branching of the quarternary tubules or the first intralobar division of the tertiary tubules. Up to this time no sign of the secreting or convoluted tubule had appeared. About these quarternary tubules, however, differentiation of the future convoluted tubule had occurred and from now on, with each succeeding division of collecting tubules, there was a simultaneous formation of convoluted tubules (including glomeruli, etc.) in the angles of the branching collecting tubules. Eventually, each branch of the collecting-tubule tree, with its corresponding convoluted tubules, was surrounded by mesenchymal tissue which formed a sort of capsule about it as a unit. In the kidney, therefore, as in the lung, liver, etc., there is a definitive lobule, which is the elementary unit of the entire excretory apparatus. This unit consists of the terminal branch of the collecting tubule, the convoluted tubules, including the glomeruli, and the surrounding endothelial and mesenchymal tissues. Owing to the lobulation, the growth and division of the collecting-tubule tree inside the lobe was much more elaborate in the 10-day than in the 9-day embryo. The distribution of the lobes in the 10-day embryo corresponded exactly to the distribution of the secondary collecting tubules. As a result, the greatest number of lobes were found composing the caudal pole, while the next greatest number constituted the cephalic pole. The lobes making up the intermediate region were evenly and regularly distributed in all planes, so that all the lobes at any given level were practically at the same stage of development regardless of their distance from the original metanephric tubule. Differentiation of the lobe always proceeded from the base towards the periphery. The most developed convoluted tubules were associated with the quarternary branches of the metanephric tubule. From this base or center the cycle of development was repeated for each generation of the renal units as we approach the periphery of the lobe towards the cortex and also towards the future columns of Bertini, not only in the longitudinal diameter but also in the transverse diameter. The development of the systems of sinuses obeyed the same general principle, that is to say, they were formed first at the base of the lobe and later at the periphery.

In conclusion, it may be stated that, although the posterior portion of the metanephric body grew and developed more rapidly than the anterior portion, nevertheless differentiation was always from the metanephric duct towards the periphery, not only in the lobes and lobules but also in the blood-vascular system and in the supporting tissue; so that even in the older embryos the most mature elements were located near the base of the lobe, while the immature structures occupied the cortical regions.

GROWTH AND DEVELOPMENT WITHIN THE EXPLANT

In cultures of chick embryos of from 8 to 10 days' incubation the evolution of all the elements making up the excretory unit of the permanent kidney could be followed in detail throughout their differentiation and development. Usually the explants contained at least one lobe with its lobules, in different stages of development according to the age of the embryo from which the piece was taken. Observations on the mesonephros were made for a comparison, but these will be described only where they differ in some fundamental way from those made upon the metanephros. The component parts of the excretory unit in cultures of different ages will be described in the order of their differentiation and development: *i. e.*, collecting tubules, nephrogenous tissue (secreting or convoluted tubules and glomeruli), and endothelium, including the blood-vascular system. The growth and development within the explant, as well as the growth extending out from the explant, were studied.

EIGHT-DAY EMBRYOS

The living explant was slightly yellowish and so transparent that the structure of the metanephros could be readily observed. The most striking characteristic of the explant was the collecting tubules, which at this age have undergone extensive growth from the metanephric ureter into the undifferentiated metanephrogenic tissue and become branched like a tree, as was mentioned when speaking of the morphology. It is in the growth of this element of the excretory unit that the mesonephros and metanephros differ most widely. In the mesonephros the collecting tubule, from the beginning of its growth from the primary excretory duct, is a single unbranched tubule, twisted upon itself, opening individually into the excretory duct, and destined to become connected with one convoluted tubule. It is coiled from the beginning, and becomes more so as it approximates maturity. The characteristic tree-like formation observed in the metanephros was never found in the mesonephros.

In the explants from 8-day embryos masses of undifferentiated metanephrogenic tissue surrounded the ends of the collecting tubules and, as there were no other differentiated elements of the excretory unit, the collecting-

tubule tree (primary, secondary, and tertiary tubules) stood out strikingly because of the marked contrast between the differentiated and undifferentiated tissue (Fig. 1). After the culture had grown for 20 hours, the explant became flattened out and attached to the coverslip, owing to the migration of connective-tissue cells, nerve filaments, endothelium, and tubules. Coincident with the migration was a division of these cells, thus forming an extensive area of new marginal growth (Figs. 3, 13, and 14). The migration of cells from within the explant to form the marginal growth left the collecting tubule intact and sharply defined (Figs. 2 and 3). The definition was aided by a single layer of highly refractive endothelial cells which completely enshrouded each collecting tubule and its branches, even covering the terminal ends of the tube (Figs. 2, 6, and 18). The endothelial cells formed a very thin sheet, through which the bases of the columnar cells forming the tubule could be seen as triangular or prismatic cellular bodies. The collecting tubules grew in a comparatively straight line from the tertiary tubules and branched out in a characteristic and regular manner, *i. e.*, always from the base towards the periphery of the lobe (Figs. 1, 2, and 8). There may be a slight, gentle curvature to the course of the growth of either the main trunk or its branches, but never was there seen the bending and intertwining observed in the tubules of the mesonephros or later in the convoluted tubules. There was a regular gradation in diameter from the tubules making up the central trunk to those constituting the peripheral arborization. The distance between the branches along the main stem was the same, although the branches came off in any plane (Fig. 2). The most common type of branch formation was dichotomous or trichotomous (Fig. 1), although there was occasionally a branching into fours. The branches always came off from the main stem at angles greater than ninety degrees, and grew in a lateral or peripheral direction. However, it must be stated that, while there was no constant direct trunk or axis of the tree growing through the center of the lobe with the branches coming off laterally, yet there was always one main branch, which maintained a general peripheralward course of growth and remained approximately the axis of the lobe (Fig. 2). The lumen of the collecting tubule was established at this age and patency was maintained by a fluid-like medium in which granules and cellular detritus were seen floating about. The lumen was bordered by a high, non-ciliated, columnar epithelium (Figs. 12 and 24). About the ends of the tubules the undifferentiated metanephrogenic tissue was observed in the form of a cap of oval cells arranged radially. These were packed together quite closely, forming a layer about four cells thick, which was, as a general rule, more dense and thick immediately over the ends of the tubules and tapered around all sides of the tubules for a short distance. Peripheral to this cap was another layer of undif-

ferentiated metanephrogenic tissue, the cells of which were less densely packed, contained fewer granules, and had no characteristic arrangement. These two regions were first described by Schreiner (1902) as the inner and outer zones, respectively, of the metanephrogenic tissue. Beyond this was undifferentiated mesenchyma, which followed the same arrangement as the cap and, by dipping down between the ends of the branches of the collecting tubules, marked out what would eventually be the lobulation of the permanent kidney (Fig. 2).

In cultures 48 to 144 hours old, the explant had become so transparent that the actual growth of the collecting tubule was easily followed. This growth took place not only by mitotic division of the cells all along the length of the tubule, but also from the ends of the tubule, the latter being the more active of the two and the more effective in increasing the length of the tubule. In the end of the tubule in which growth and branching were about to take place, there occurred a symmetrical bulbous or ampullar formation, due to a general proliferation and change in shape of the cells making up the the ends of the tubule. In areas of active proliferation and rapid growth, there seemed to be a metamorphosis always to the spherical type of cells with a subsequent reversion to the adult type. As a result of this proliferation, there occurred, coincidentally with the bulbous expansion, bud-like projections from the blind end of the tubule. Usually these projections were situated at opposite poles of the tubule; occasionally there were three or even four of them. Their rate of growth was approximately the same. At first they appeared to be growing out horizontally to the cortical surface of the lobule; they soon altered their course, however, one growing almost perpendicularly and continuing in a general way the peripheralward growth of the tree, while the other or others tended to grow out more horizontally, making up the surrounding arborization. These bud-like projections were at first solid sprouts, but by continued proliferation and reshaping of the spherical cells into a columnar type the bud increased in length, breadth, and thickness and by a rearrangement of the cells a lumen was formed, which in a very short space of time extended almost the entire length of the sprout; thereafter, the lumen formation progressed hand-in-hand with the growth of the tubule. This phenomenon was of a different type from that described by Dr. Sabin (1920) in the formation of the lumen of the blood-vessels, for in the collecting tubule no vacuolization of the cells took place; on the other hand, a small but gradually widening cleft within the sprout, was formed by a rearrangement of the cells and a transformation in shape to that of the columnar type. The new collecting tubule branches continued to grow with a simultaneous formation of a lumen, until a certain length had been attained, when a new branching occurred. The endothelial sheet covering the tubule proliferated and grew simultaneously

with the growth of the branches, and the collecting-tubule tree was thus covered throughout with a shroud of endothelium. The undifferentiated metanephrogenic tissue was plainly visible in the cultures and its growth and development were closely associated with that of the collecting-tubule tree. Felix (1912) described this as forming a circumferential sheet which surrounded the ends of the ingrowing collecting tubules and was pushed peripheralward by the growth of the tubules after each succeeding layer of newly formed convoluted tubules and glomeruli had been formed. This, however, was not found to occur in the chick. Here the undifferentiated metanephrogenic tissue surrounded each individual collecting tubule that grew into the lobe; as the tubule branched, this metanephrogenic tissue was split up, some of it being carried forward by the growing branches, the rest remaining behind in the angles between the branches. The portion of the metanephrogenic tissue carried forward over the end of the tubule extended to cover the new branches of the tubule, partly by proliferation and partly by a peripheralward migration, as was evidenced by a slight distortion of the cellular mass.

The deposition of the undifferentiated tissue in the angles of the branching tubules was found not only in the cultures and spreads but also in the sections of the metanephros (Figs. 10, 13 and 16), and it was from this tissue that the future convoluted tubules and glomeruli always developed. This constant relation of the undifferentiated metanephrogenic tissue to the ingrowing collecting tubule was especially striking in the cultures. No matter to what extent the collecting tubule may have grown inside the explant or out into the margin, as will be shown later, there was always the same relation maintained between it and the undifferentiated metanephrogenic tissue (Figs. 8 and 10). Since the convoluted tubules always developed in the same position with relation to the collecting tubules, and always from the metanephrogenic tissue of the inner zone and never elsewhere in the mass of undifferentiated tissue, it may, I think, be deducted that the metanephrogenic tissue of the inner zone is made up of cells that are predestined to become the anlage of these convoluted tubules. This anlage always appeared as a mass of undifferentiated tissue, quite distinct from the surrounding nephrogenous tissue of the outer zone and the collecting tubule (Figs. 10 and 15). The cells constituting the inner zone, which formed a cap-like covering over the blind end of the ingrowing collecting tubule, were at first quite similar in form. However, within 24 hours, in cultures of the 8-day embryos, morphological changes, and also changes in the general arrangement of the cells, were observed. The cap became sharply defined by three and often four layers of cells which completely ensheathed it (Figs. 15 and 18). The cells making up these layers were rather long and narrow, approximating the endothelial

cell in shape, and stood out in marked contrast to the adjacent tissue. Between the two outermost layers appeared a space, which likewise encircled the anlage and served to define it still more sharply (Figs. 16, 18, and 24). Simultaneously with the differentiation of this endothelial sheath, there occurred not only a change in the morphology of the cells making up the cap but also a change in the form of the cap itself. The cells proliferated rapidly, becoming more spherical in shape and somewhat less granular. There was also an increase in the size of the individual cells. Thus there was formed, as it were, a solid central core of spherical cells approximating the epithelial type (Figs. 15, 16, 18, and 19). The difference in the polarity of these two distinct types of cells was quite striking. In the spherical cells there was a tendency toward a radial arrangement about a central core. The axes of these cells were perpendicular to the axis of the central core, while the axes of the endothelial-like cells about the periphery of the central core were at right angles to the axes of the spherical cells (Figs. 16, and 18). In twenty-four hours' growth, before any suggestion of tubule formation, there was thus differentiated *in situ* two distinct types of cells, endothelial and epithelial, from one common mass of undifferentiated tissue. Furthermore, the entire anlage had become surrounded by a space lined with endothelium (Fig. 18).

Concomitantly with the differentiation of these two types of cells, the inner cell mass, composed of spherical epithelial-like cells, was observed to become shaped like an inverted comma (Figs. 19 and 20). (This comma shape was first mentioned by Ribbert in 1899.) The head of the comma was always to the side of the ingrowing collecting tubule, while the tail covered its blind end (Figs. 16 and 19). By continued growth the comma-shaped mass increased in size and underwent a gradual transition into a solid S-shaped tubule or core. This transition required about 24 hours. At first its differentiation and growth were uniform throughout, that is, the tail end of the comma appeared quite as early and was as far advanced as the head-pole (Fig. 19). However, during the transition the most rapid growth was seen to occur in the head pole of the comma, or what I shall call the distal pole, where the spherical cells became arranged in what appeared to be a solid, sphere-like dilatation (Fig. 21). Immediately above this sphere the growth was almost as rapid, causing an angulation which resulted in the formation of the first curve of the future S-shaped tubule (Fig. 21).

Differentiation beyond this stage was not observed in the metanephrogenic tissue of the inner zone in the 8-day embryos. In the outer zone, however, growth and differentiation did occur. The future connective-tissue septa appeared at the periphery of this outer zone of the metanephrogenic tissue and with their appearance there were laid down the interlobular septa constituting the micro-

scopic lobulation. Throughout the growth of the collecting-tubule tree, lobulation was observed to keep pace with the formation of branches of the tree.

Nothing has yet been said concerning the region about the main stems and branching collecting tubules. Concomitant with the growth and development of the lobule, there was constantly left behind, in the path of differentiation and peripheralward growth, a cellular mass of tissue which surrounded the trunk of the collecting-tubule tree for that lobule. This tissue constituted a supporting framework containing the blood capillaries, for the developing excretory unit. Simultaneously with the differentiation of the excretory unit, and well advanced before the appearance of the convoluted tubules, there was formed progressively a system of sinuses. As stated under the description of the morphology, the process of differentiation and development of endothelial sinuses was first observed in the undifferentiated metanephrogenic tissue between the bases of the primary collecting tubules in the 6-day embryo. The presence of a sinusoidal circulation in the mesonephros was first mentioned by Minot (1892), but nothing was said about the existence of a sinusoidal circulation in the metanephros or the method of development of these sinuses. In cultures of the 8-day embryo all stages of development of the sinuses could be seen. The most fully developed were near the base of the lobe, while the least developed ones were found about the anlage of the excretory unit, toward the periphery. This formation of sinuses followed, so to speak, in the wake of the growing collecting-tubule tree (Figs. 1, 13, 14) and, as in the differentiation and development of all the other elements of the lobe, the progression was always from base to periphery. In the cultures the sinuses were sharply defined from the surrounding tissue and they also were readily seen in fixed sections, although much shrunken and distorted (Fig. 16). About the base of the lobes the sinuses were quite large and filled with a fluid medium, in which blood elements were commonly found (Figs. 5, 13, and 17). The walls of the sinuses consisted of a single layer of endothelial cells, which at times were in apposition to the endothelial layers covering the tubules, and at other times were connected with these layers of endothelium by endothelial sprigs or offshoots (Figs. 12, 16, 17, 24). The larger sinuses connected freely with each other and also with a capillary network of the lobe, so that in an embryo of this age there were two distinct blood-vascular systems, capillary and sinusoidal. Blood-islands were commonly seen attached to the inside of the walls of the sinuses and from these hemoglobin-containing cells, as well as white blood-cells of lymphocyte type, were differentiated. Sinuses also were differentiated *in situ* from the undifferentiated tissue making up the bulk of the metanephric body. These sinuses had their origin in the vacuolization of cells quite similar to angioblasts. These cells were rather

conspicuous, due to the fact that there were always two and sometimes three in juxta-position, making them appear as deeply pigmented multinucleated giant cells, joined together by very minute protoplasmic processes (Fig. 6). In each cell there appeared a vacuole, which constantly enlarged until there remained only a rim of cytoplasm and an eccentrically placed nucleus. The vacuolization of these cells was complete in 24 hours and fusion of the two or three original cells occurred, resulting in a small space surrounded by flattened out cytoplasm and eccentrically placed nuclei. This space increased in size by multiplication of the flattened-out cells and was held patent by a fluid medium. These sinuses were of different shapes and sizes and were connected with each other, as well as with the capillary network, and with the endothelial spaces about the anlage of the convoluted tubule, by means of direct sprouting of the larger sinuses. Throughout the entire life of the culture differentiation of capillaries *in situ* and direct growth after differentiation by sprouting was observed. Thus, the sinusoidal and the capillary systems develop *in situ* at the same time from a totally undifferentiated mass of tissue making up the bulk of the metanephric body. The two types of circulation were very similar in their differentiation and development.

In old cultures the macrophages appeared to ingest and digest tissue left behind in the wake of the developing lobe (Fig. 14) as well as injured tissue. Inside these macrophages red blood-cells and other forms of cellular detritus were observed. The macrophages were much more numerous in the base of the lobe.

NINE- AND TEN-DAY EMBRYOS

In the explants of the metanephros, made from 9- and 10-day embryos, one had, of course, a more completely developed excretory unit to begin with than in the explants from 8-day embryos. Notwithstanding the fact that there were growth and differentiation in cultures from the latter, these were always much retarded as compared with an embryo of corresponding age. In the explant of the metanephros from 9- and 10-day embryos, the collecting-tubule tree had become much more branched and complex throughout the lobe. The main trunk had increased not only in length but also in diameter, and its primary branches had already divided into secondary, tertiary, and quaternary branches. The dichotomous branching of these tubules continued with a lobulation or division of the lobe into smaller lobules by the rearrangement of the mesenchymal septa, corresponding to the branching tubule tree. The sinuses and blood-vascular elements formed and grew just as in cultures from the 8-day embryo, so that the essential difference between the growth of the explants in the two series lay in the differentiation and development of the secreting tubules.

All stages, from completely differentiated to entirely undifferentiated metanephrogenic tissue, could be studied

in the explants of 9-day embryos, as there was constantly occurring a repetition of the cycle of differentiation and growth of the complete excretory unit towards and in the future cortical portion of the lobules. Between the 9th and 10th day of incubation seemed to be the optimum age for a study of the development of the metanephros. Whether this was due to the fact that metanephrogenic tissue was of a greater vitality than at 7 and 8 days, or whether the undifferentiated cells were more nearly on the verge of differentiation and therefore less affected by the change in environment, I cannot say; in any event, within twenty-four hours all stages of differentiation from a comma-shaped body to an S-shaped tubule could be observed. The main areas of growth during the transition were the sphere-like group of cells at the distal pole and, immediately superior to this, the portion which was to form the first knuckle of the future S-shaped tubule (Fig. 21). At these two points proliferation was most active. The continued growth of the sphere at the distal pole, immediately under the rapidly proliferating first curve, caused a slight flattening of the sphere with a subsequent bulging about the sides and to some extent the ends of the overhanging knuckle of the first-formed curve. The growth of the sphere and that of the large knuckle (first curve), being parallel and in the same direction, caused an acute flexion of the solid core immediately posterior to the sphere, thus forming the second curve of the S (Fig. 22). Owing partly to the relatively slow growth of the cells making up this second curve, and partly to the pull of the rapidly growing sphere and first curve, the second curve became very small in diameter and acutely flexed (Figs. 6, 23). The solid S-shaped mass, therefore, developed *in situ* and not, as heretofore thought, from a sphere into an S-shaped tubule. The entire solid core developed from cells already present, and assumed an S-shape because of more rapid growth in some regions than in others.

The endothelial sheath, together with the sinus formation, progressed hand-in-hand with the development of the S-shaped core, i. e., the S-shaped core was completely covered by a layer of endothelial cells and in addition to this the entire anlage was completely surrounded and delimited by a space or sinus lined with endothelium (Figs. 12, 16, 24). The core was thus sharply outlined. A marked difference in the refractivity of the endothelial and epithelial cells further aided in the definition of the tubule so that it was easy to distinguish changes taking place within the tubule from those in the tissue without. The close approximation of the glomerular pole to the under surface of the first curve of the S might give the false impression of a cleft formation, as stated by Ribbert (1899) and Huber (1905) instead of a solid core doubled upon itself (Figs. 21, 22). The potential space between the distal pole and the under surface of the first

curve contained the endothelial layers covering the solid tubule and those forming the wall of the sinus (Figs. 17, 21, 24), so that the bud of an endothelial sac was thus placed between the glomerular end of the tubule and the under surface of the first curve. This slight indentation, however, approximated in no way the cup formation described by other investigators, notably Huber, Schreiner, and Felix.

After the comma-pattern had developed into an S-shaped tubule, there was a continued growth throughout the entire tubule. Proliferation of the cells in the glomerular end occurred more rapidly than elsewhere in the S-shaped tubule until that end became dilated and bulbous, forming a tuft of epithelial cells (Figs. 2, 6, 11, 22). At this stage no capillaries or blood elements were ever observed in this tuft, all the cellular proliferation being inside the endothelial sheath, and therefore within the tubule. The cells causing the dilatation of the glomerular end were at first all more or less round; however, a differentiation of these cells took place *in situ* along with their continued proliferation, resulting in the formation of flat, endothelial-like cells, and spherical epithelial cells, thus constituting the pre-glomerular tuft (Figs. 17, 23). The endothelial cells, because of their shape and greater refractivity, could be traced winding about amongst the epithelial cells (Figs. 23, 24). The latter, however, formed the greater part of the cell mass producing the dilatation of the glomerular end of the tubule. By 72 to 96 hours, collapsed endothelial spaces could be seen winding through the epithelial tuft and everywhere covered with epithelial cells (Fig. 24). Thus the presence of endothelial cells intimately intermingled with epithelial cells was observed in the bulbous glomerular end while the future secreting tubule was still in a solid state and as yet no connection with any type of circulatory system could be seen. No evidence of a growth from the endothelium covering the exterior of the tubule down into the glomerular end was observed. The cellular proliferation in the glomerular end proceeded rapidly, the dilated bulbous end being completely filled and much distended in comparison with the remainder of the tubule. The curve of the tubule immediately proximal to the glomerular end became reduced to a very small tube whose walls were formed by a rather low cuboidal epithelium one cell in thickness, covered by an endothelial sheath. In the loop of the S immediately proximal to the glomerular end the formation of a lumen was first observed (Fig. 23). This followed the same course as in the collecting tubules, *i. e.*, the lumen formed by a rearrangement and separation of the cells and not by vacuolization, as was observed in the vascular system.

The cells abutting against the lumen were the first ones to become cuboidal in shape. The rearrangement of the cells about the lumen progressed distally and proximally with about equal rapidity. The lumen formation began

about the inferior half of the spherical tuft of cells, then progressed to the superior half, until the central tuft was completely separated from the walls of the glomerular end of the convoluted tubule, except at one pole, usually distal to the point of transformation of the secreting tubule into the glomerular end (Figs. 6, 23, 24).

This lumen formation in the glomerular end was the anlage of the capsule of Bowman, the cells bordering the lumen constituting the layers of the capsule (Fig. 24). The flat epithelial cells formed the parietal layer, while the cuboidal cells made up the visceral layer. Immediately before the formation of the lumen the spherical tuft of cells attained its maximum size, decreasing from that time on until the glomerulus began to function, when it again enlarged. With the completion of the lumen formation and the changes in shape of the bordering cells forming the anlage of Bowman's capsule, the appearance of an invagination of the remainder of the spherical tuft was obtained. However, the etched-like outline of endothelial cells enabled one always to separate that which occurred inside the tubule from that which occurred outside. Any invagination or cup formation that involved the entire glomerular end of the secreting tubule would of necessity cause a disturbance of the contour outlined by the ensheathing endothelial covering. If sections of merely the cuboidal cell layer of Bowman's capsule constituting the most distal margins of the glomerular end of the tubule are reconstructed, then one obtains an incorrect impression of a tubular invagination.

A connection was now established between the newly formed space or sinus surrounding the secreting tubule and the larger sinuses already formed. This connection was by means of direct sprouting off of endothelial strands from the larger sinuses which became confluent with the sinuses differentiated *in situ* about the anlage of the secreting tubule. Thus the endothelium-lined space between the superior surface of the glomerular end and the second curve of the S-shaped tubule was in direct communication with the larger sinuses. A continuation of endothelium was then established from the endothelial elements within the glomerular epithelial tuft through the sinus about the secreting tubule by way of endothelial sprouts into the larger sinuses about the collecting tubules (Figs. 2, 24). The endothelial sprouts connected the glomerulus with the sinuses always through the attachment of the glomerular tuft of cells to the wall of the tubule; that is, at the point where the capsule of Bowman was reflected, which usually lay distal to the urinary pole. At this point the visceral and parietal layers of the capsule were confluent. In the older cultures it was found that the sinuses became connected with the capillaries that developed in their neighborhood.

Hematopoiesis occurred in the spherical tuft of the glomerular end with the formation of red blood-cells, plasma, and white blood-cells having the appearance of

lymphocytes (Figs. 17, 23, 24). The formation of the blood-elements was usually first noted in that portion of the tuft adjoining the secreting tubule and progressed from this point throughout the glomerular portion. It occurred at about the same time the connection with the sinuses took place. It must be borne in mind, however, that in the cultures no circulation was present. At no other point in the entire excretory tubule did hematopoiesis occur, a fact which also indicates the endothelial nature of the cells inside the spherical tuft. Lumen formation by thinning out of the cells, characteristic of the blood-vascular system elsewhere, was found to obtain in the glomerular tufts. The blood elements made the definition of the endothelial channels much more pronounced and the latter could be traced with ease, winding among the epithelial cells and covered by a single layer of these cells.

After the differentiation, the convoluted tubule continued to grow in exactly the same manner as the collecting tubule, that is, by proliferation of the cells along the entire tubule or at the end nearest the collecting tubule. The convoluted tubule grew always toward the collecting tubule, until finally the proximal end of the former abutted against the side wall of the latter. This contact usually, though not always, was in the immediate region of a newly formed branch of the collecting tubule. The anastomosis of the convoluted and collecting tubules could be observed in cultures of the 9-day embryos after 24 hours' growth. In general, it required about that length of time to effect complete continuity of the convoluted and collecting tubule lumina. The continued growth of the secreting tubule against the wall of the collecting tubule exerted sufficient pressure to indent the latter and cause a distortion of its constituent cells (Fig. 23). The secreting tubule grew into the collecting tubule at an angle of about forty-five degrees. The cells in the wall of the collecting tubule formed a sort of arciform arrangement about the ingrowing blind end of the convoluted tubule. There was a rapid proliferation of the cells constituting the latter and the cells reverted to a spherical type, as was observed in the growth of the collecting tubule. As a result of this proliferation, the end of the convoluted tubule protruded like a wedge through the wall of the collecting tubule into the lumen. This was not due merely to pressure from the outside against the wall of the collecting tube; there was an actual growth of the convoluted tube inside the wall of the collecting tube, the cells dividing and making room for the increased number. An interruption in the continuity of the wall of the collecting tubule was thus effected (Fig. 24).

Lumen formation in the secreting tubule followed upon the proliferation of the proximal or blind end, so that about two hours after the secreting tubule had grown through the wall of the collecting tubule the lumina of

the two had become continuous (Fig. 24). This continuity was proved by the coursing of fluid containing granules back and forth from one tubule into the other. After the anastomosis of the convoluted and collecting tubules, there was continued growth of the former throughout its entire length, as evidenced by mitotic figures. The greatest growth activity, however, occurred in the convoluted portion next to the collecting tubule, which in the adult kidney would correspond to the distal pars convoluta.

DEVELOPMENT IN MARGINAL OUTGROWTHS

The outgrowths from explants of the metanephros (of embryos of 8 to 10 days inclusive) were made up chiefly of four types of tissue—mesenchyme, renal epithelium, endothelium, and nervous tissue. The marginal growth began immediately after implantation. In from two to four hours after explanting, proliferation of mesenchymal cells about the margin and growth of nerve-fibers into the marginal zone could be made out. Growth of the four elements of the marginal zone increased steadily throughout the life of the culture up to 5 days. In the beginning it consisted of only a thin sheet about one cell in thickness. The most advanced edge of the outgrowing margin always remained quite thin and attenuated, but as the growth increased in width, so did it increase in depth. The different elements composing the marginal outgrowth proliferated and grew with different degrees of rapidity, but there was little difference ultimately in the extent to which they grew.

Usually the first tissue to grow was the mesenchyme; it was also the most hardy and prolific of all the tissues (Fig. 5). In appearance, these cells were typical fibroblasts connected with one another by long branching processes, which were direct extensions of the cytoplasm (Fig. 7). At first there was no definite arrangement of the mesenchyme cells; they simply grew out in a flat spreading sheet with a centripetal tendency (Figs. 4, 14). However, after 48 hours, when the growth near the explant had become thicker in different areas, definite patterns and arrangements of cells could be seen, although the attempt of the cells to assume their normal formation was often abortive and resulted in bizarre disturbances of organization (Fig. 14). This interference with the tissue in its effort to acquire its predestined form was due, no doubt, to surface tension and adhesion to the coverslip.

The renal epithelium grew out readily, but like the mesenchyme, in different forms in the various cultures. Proliferation and growth began immediately at the margin. Arising from the excretory unit (the collecting tubules, convoluted tubules, glomeruli, and undifferentiated nephrogenous tissue) growth proceeded in three fundamentally different ways: (1) with no particular formation, as in a flat sheet (Fig. 5); (2) as a perfectly organized element, carrying with it undifferentiated tissue, which later differentiated in the marginal outgrowth

(Figs. 8, 9); (3) out into the margin where an abortive attempt at formation or organization was made (Figs. 3, 4, 14). Wherever there was an end of a collecting tubule or convoluted tubule near or immediately at the margin of the culture, there was always an outgrowth of renal tissue, which appeared quite as early and grew as rapidly as the mesenchymal tissue. When growing out hand-in-hand, as it were, with other tissue of the margin, the renal tissue always appeared as a flat sheet of cubical cells closely associated with one another. This sheet was usually one cell thick and often bounded on all sides, except towards the periphery of the growth, by outgrowing mesenchymal and endothelial cells. In these flat outgrowths there was never any attempt at organization or differentiation; the outgrowth consisted simply of a multiplication of already differentiated cells.

In the event that a well-advanced marginal growth had preceded the outgrowth of renal tissue by a few hours so that there was some depth of cells into which the renal tissue could grow, then the outgrowth and differentiation occurred exactly as they did inside the explant. In other words, the collecting tubule grew into the marginal zone as a tubule intact, just as the collecting tubule grew inside the explant, carrying with it undifferentiated nephrogenic tissue which went on to complete differentiation in the margin (Figs. 8, 9, 11). The excretory tubule maintained the same relation to the collecting tubule, with regard to position and growth, that it maintained inside the explant: when it differentiated, it always did so *in situ*, i. e., in the region or at the site of the angle formed by the branching of the collecting tubule, afterward becoming S-shaped by direct growth of the already differentiated cells. There was never a long, straight, direct outgrowth of the convoluted tubule such as there was in the collecting tubule.

When the marginal growth of cells was not thick, the attempts at organization were abortive, although differentiation occurred. In all marginal growths there was a certain point beyond which the entire margin grew out flat and "went wild," that is, all formation or organization was lost and the individual elements broadened out into a flat sheet. This transition could be followed in most of the cultures that survived long enough.

The third element in the marginal growth was the endothelium. As was the case with the renal tissue, the endothelium grew out in different ways. In the first place, there was a sheet-like growth, with greater intervals between the individual cells, however, than occurred in the mesenchyme or renal tissue (Fig. 3). In fixed preparations the endothelial cells were fibrillated and connected by long branching processes. No formation of blood-vascular elements was seen in this type of outgrowth. On the other hand, capillaries grew out in arciform loops from other capillaries already formed in the explant. The growth of these could be watched in detail.

The development *in situ* of sinuses, blood-islands and capillaries occurred in the marginal outgrowth wherever the necessary undifferentiated tissue had been carried out from the explant. Among the tissue of the explant and marginal outgrowth, at all ages, long nerve-fibers, also ganglia, were sometimes observed. In the marginal growths, therefore, there occurred differentiation and organization of the renal parenchyma, as well as of the other basic tissue elements making up the renal body.

Differentiation, followed by a marked distortion due to the attempt of the cells to assume their usual organized positions, afforded an excellent opportunity for study. One could find places in which epithelial cells had differentiated in small flattened circular areas. Among these were highly refractive endothelial cells, intimately associated with the epithelial cells, being interwoven among them in exactly the manner noted in the development of the glomerulus. As this association of endothelium and epithelium was not observed anywhere else in the marginal growth, although there were many outgrowths of renal epithelial cells, the logical inference is that these small translucent circular areas were probably made up of cells destined to develop into the glomerulus.

DISCUSSION

Most observers agree on the growth and division of the collecting-tubule tree and the coincident splitting up of the nephrogenous tissue, thus affording a cap or covering for each branch of the collecting tubules. As far as I have been able to find, however, none of the previous observers have described the endothelial covering of these tubules, although several figures given by Herring show it.

In regard to the nephrogenic tissue, all observers agree that an S-shaped tubule is formed from a renal vesicle situated in the angle made by the collecting tubules. Ribbert (1899), was the first to call attention to the fact that a comma-shaped body preceded the formation of the tubule. Huber (1905, p. 17, Fig. 2) presents a drawing of a section of a human embryo, showing a collecting tubule, on each side of which a comma-shaped body is clearly defined. In the inferior pole of each of these bodies can be seen a renal vesicle, which Huber states differentiates out *in situ*. He does not specifically refer to the main body or the tail of the comma except to say that the renal vesicle becomes separated from the nephrogenous tissue and becomes bordered by columnar cells. The S-shaped tubule is then formed by constant growth and elongation of the renal vesicle produced by active proliferation and mitotic division of the cells. In the figure referred to above the arrangement of the cells will be found to coincide exactly with the description of my observations, the cells making up the tail and body of the comma being arranged with their axes perpendicular to the axis of the central core. Huber mentions only the sphere of cells at the lower pole and disregards

the remainder of the comma. He, as well as all other previous observers, admits that the renal vesicle develops *in situ*, but though they show in all their figures that the remainder of the comma also undergoes a definite rearrangement of cells and differentiation, still nothing but the spherical end of the nephrogenous tissue has been heretofore considered. In my observations the entire comma-shaped mass was seen to differentiate *in situ* from the undifferentiated cap of metanephric tissue and to form the S-shaped tubule.

In the formation of the convoluted tubule, from one type of tissue there are differentiated, therefore, cells of very diverse biological activity. These inherent differences are evidenced in the embryo by a difference in the growth rapidly in the several regions of the tubule anlage, and foreshadow the different functions of these respective areas in the adult.

The *modus operandi* of the union between the secretory and collecting tubules has never been fully described, although Schreiner hypothesizes that it occurs in given regions of the collecting tubule which are recognizable by the presence of mitotic figures. This theory is not surprising, in view of the fact that previous observations have been made on sections. In the study of the living tissue, it was possible actually to watch the uniting of the convoluted and collecting tubule, and it was seen that the convoluted tubule definitely grows into the collecting tubule.

Another very interesting fact, brought out in the cultures, was that, no matter to what extent undifferentiated tissue was displaced from the explant, it assumed within certain limits its predestined shape and activity. Nephrogenic tissue developed into secreting tubules and always became convoluted, while the reverse was true of the collecting tubules, which always grew in a comparatively straight line, regardless of the absence of pressure from surrounding structures or other physical factors that occur normally in the embryo. This, it would seem, shows that this tissue has been given a stimulus to assume a certain form and activity which will be accomplished, unless the tissue reverts, by a state of extreme activity of growth, to a spherical type of cell. This was shown in the development in the explants and also in the growth comprising the margin.

Bowman's capsule is formed from the end of the secreting tubule, which previous investigators claim invaginates to receive the coincident ingrowth of capillaries to form the glomerulus. While it might be possible to arrive at such a conclusion from an examination of sections, the study of the living tissues, either in spreads or tissue-cultures, shows that it is not the correct one. In the first place, the endothelium that differentiates *in situ* around the convoluted tubule, coincidentally with the growth and differentiation of the latter shows, from the earliest appearance of the bulbous enlargement of the

glomerular end, that the wall adjacent to the overhanging first curve of the S-shaped tubule, remains intact throughout the development of the glomerulus. In the second place, in the earliest stages the dilated bulbous end of the glomerulus contains no lumen, but consists of a solid mass of spherical cells, which in appearance resemble epithelial cells. By a rearrangement of these cells there is a separation which results in the formation of a lumen within this cell mass, which leaves the epithelial cells on one side and the mass of epithelial and endothelial cells on the other.

The epithelial cells forming the peripheral wall of the tubule, however, become quite flat and very much elongated, approximating somewhat the endothelial type of cells and thus may have been mistaken for endothelial cells by other observers. According to previous writers the spherical end of the convoluted tubule increases steadily in size until the invaginating cup reaches its maximum, whereas in my observations the spherical tuft reached its maximum size before the completion of the lumen. Immediately upon completion of the lumen, the visceral and parietal layers of the anlage of Bowman's capsule are formed *in situ* within the tubule from undifferentiated spherical cells. In fixed sections, these layers of Bowman's capsule show up very clearly and it is easily seen how other investigators, considering these walls to be the boundaries of the tubule, would obtain the impression of an invaginating cup. In the fixed sections, however, interrupted stages only can be observed, whereas, in the living, the entire process of lumen development can be followed as a whole.

Concomitant with the formation of the lumen and differentiation of the cells adjacent thereto, there is differentiation of the cells within the spherical tuft, resulting in the development of endothelial cells in the most intimate association with the epithelial cells. These endothelial cells later form channel-like tracts which are covered by a single layer of epithelial cells. Hematopoiesis in the spherical tuft occurred in the cultures before any demonstrable connection with the sinuses could be seen, and it must be borne in mind that there was no circulation of blood elements whatever within the explant. At a later stage the connection of endothelial channels with the surrounding sinus was always observed to take place through the small neck of tissue connecting the spherical tuft of cells with the wall of the tubule, a communication with the ingrowing branches from the aorta being established still later.

The idea that endothelium and blood develop *in situ* was first suggested as a speculation by Herring. To him it seemed possible that the capillaries might develop *in situ* and not as ingrowths from the dorsal aorta during the formation of the glomerulus. Schreiner and Huber, on the other hand, claimed that the glomerular tuft is formed entirely by an ingrowth of pre-existing capillaries.

The later work of Sabin in blood-vessel formation, in which she states that not only the endothelium, but also the blood of the early vascular system arises *in situ*, suggests the possibility that, in other regions where primitive tissue exists, the vascular arrangement may also develop *in situ*. Certainly the observations on the development of the glomerulus in tissue cultures, where the mass of primitive mesenchyme from which this organ develops is entirely isolated from any circulation, show that not only epithelial cells, but also endothelial cells and blood-cells, differentiate *in situ* from the mass of undifferentiated spherical cells within the glomerular end of the convoluted tubule which goes to form the glomerulus. The intimate relation of the glomerular capillaries and the epithelial covering is thus explained, for it is quite difficult to figure out how this epithelial covering of each capillary could be acquired by the mere ingrowth of the capillaries into an epithelial sac. In addition to this, the sinuses also develop *in situ*, as could be seen not only from a study of spreads and tissue cultures but also in fixed sections. This development of the blood spaces *in situ* might have been surmised by Colberg (1863) from injections. He injected the blood-vessels of the kidney and found that some of the glomeruli were injected while others were not. The latter he called pseudo-glomeruli. These were undoubtedly glomeruli at an early stage of development before connection with the peripheral circulation was established. The same opinion was expressed by Herring in 1900. In 1911 Jeidell studied three injected pig embryos and found that a rich capillary network was formed in the mesenchyme surrounding the renal anlage from the inferior mesenteric and middle sacral arteries, the drainage occurring into the post-cardinal vein, inferior mesenteric veins, and capillaries of the Wolffian body. None of these injections, however, so far as I can make out from her paper and figures, penetrated farther than the primitive capsule. She concludes that the renal anlage is supplied by this means but does not state how the blood enters from the surrounding mesenchyme. In a study of human embryos of different ages, Kelly and Burnam (1914) claimed from injections that the development of the permanent blood supply of the kidney occurs when that organ reaches its definitive position and that, previous to this time, the circulation is maintained by a communication of the capillaries of the kidney with capillaries of the surrounding mesenchyme. Thus, in the ascent of the kidney, the capillaries which are left behind atrophy, while new ones form about the superior pole. At the end of seven weeks a connection with the aorta is established. Before this, no injection of the kidney could be made. Somewhat the same phenomenon occurred in the chick. Previous to complete development of the secreting tubule, I was unable to reach the sinuses or glomeruli of the metanephros by way of the peripheral circulation by injections of India ink into the heart, whereas the liver

and mesonephros became so heavily injected as to appear entirely black. I think it can be concluded, therefore, that in the incompletely developed metanephros there is no continuity of or direct connection with the peripheral circulation; that there is a sinusoidal circulation, which by continued growth and constant sprouting is diminished and transformed into a capillary circulation, has been shown above. The presence of blood elements and a fluid medium inside these sinuses insures a vehicle to carry oxygen or nutritives to the developing renal body. The nourishment of the kidney in its early stages of development is therefore somewhat analogous to the nourishment of a fetus by way of the placenta.

Sabin (1920), from studies of the blood-vascular system of living chick embryos up to four days, concludes that the various white blood-cells have different origins. The lymphocytes and monocytes arise from the endothelium lining the capillaries, while the granulocytes arise from the mesenchyme cells and migrate into the lumen of the blood-vessels. A particular study was not made of the exact origin of each type of blood-cell, but it was seen that not only the capillaries, but also the sinuses, often were filled with lymphocytes which had arisen from the endothelial walls, and in many instances, granulocytes were observed scattered among the mesenchyme cells, as though they had differentiated in that region.

CONCLUSIONS

1. In the above study of the development and growth of the metanephros of the chick embryo it is found that a continuous sheet of endothelial cells entirely surrounds the collecting and convoluted tubules.
2. The convoluted tubules differentiate *in situ* from a comma-shaped mass of undifferentiated nephrogenic tissue. The S-shaped tubule is developed out of the comma-shaped mass by different degrees of growth in different areas of the mass, and not by elongation of a renal vesicle.
3. The glomerulus, including its capillaries and blood elements, differentiates *in situ* from an undifferentiated cellular mass which completely fills the distal end of the convoluted tubule. There is no cup formation with subsequent ingrowth of capillaries.
4. The blood system surrounding the metanephric tubules is at first principally sinusoidal, as in the pronephros and mesonephros, being later almost entirely replaced by a capillary system. These sinuses are not at first in direct continuity with the capillaries of the peripheral circulation, but circulation takes place by diffusion between them and the capillaries.
5. The convoluted tubules grow directly into the intact collecting tubules.
6. In tissue cultures of the metanephros there does not occur a dedifferentiation of tissue but an active growth and differentiation takes place not only in the explant but also, within certain limits, in the marginal outgrowth.

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EXPLANATION OF FIGURES

PLATE 1.

FIG. 1.—16 mm. lens. Seven days' growth of metanephros of 8-day chick embryo. Extensive growth of the collecting-tubule tree not only within the explant but also out into the marginal growth. The collecting tubules have grown into the margin as intact tubules, branching in the characteristic dichotomous manner, the line of growth being a relatively straight one. In this figure the branches are all intralobar, the main stem to the left of the figure being a quarternary branch. The large clear areas between the basic branches are the sinuses; smaller ones are seen toward the periphery. Throughout the culture there are several displaced collections of undifferentiated nephrogenous tissue. The margin of the growth appears in the lower right hand corner of the figure.

FIG. 2.—16 mm. lens. Seven days' growth of the metanephros of a 10-day chick embryo. Entire excretory unit completely differentiated inside the explant. Union between the convoluted and collecting tubules has occurred in most instances. The main stem of the collecting tubule here is made up of two tertiary tubules. In the upper portion of the figure there are some convoluted tubules which have grown more nearly in a straight line than usual. All of the convolutions show typical dilation of

the glomerular ends. The glomeruli here are quite small. Numerous macrophages can be seen throughout the culture clearing up the remaining undifferentiated tissue by phagocytosis.

FIG. 3.—16 mm. lens. Seven days' growth of metanephros of 8-day chick embryo. Marginal outgrowth of collecting tubules on the right. The tubules grew out intact within the thicker region but became flattened out into a sheet-like growth in the more attenuated portion of the margin. At different points in the marginal outgrowth can be seen endothelium composed of cells larger and flatter than the mesenchyme cells, which make up the greater part of the outgrowth. Owing to the heavy staining, the structures inside the explant cannot be made out. Formed sinuses in the explant at the left are well shown about the base of the collecting-tubule outgrowth.

PLATE 2.

FIG. 4.—16 mm. lens. Six days' growth of metanephros of 8-day embryo. Extensive outgrowth of formed collecting tubule into margin, flattening in the attenuated edge of the culture.

FIG. 5.—16 mm. lens. Seven days' growth of metanephros of 8-day embryo. Extensive outgrowth of renal epithelium from collecting tubule in the explant. There is a lumen in this for a short distance in the marginal growth. The endothelium can be seen about the tubule and is quite characteristic in this mode of outgrowth. This endothelial growth originates from the endothelial covering about the tubules and presents the same type of outgrowth that occurs from endothelium elsewhere.

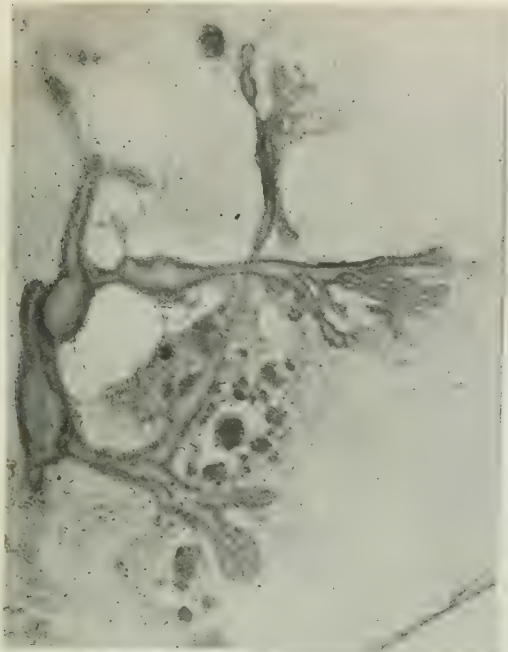
FIG. 6.—4 mm. lens. Four days' growth of metanephros of 9-day chick embryo. Iodine vapor. Comparatively straight collecting tubule into which the convoluted tubule has grown. The point of union is shown in the lower left hand corner of the figure, where the convoluted tubule appears to be constricted. The tubules are sharply defined by the characteristic etched-like endothelium. The lumen formation in the glomerulus has been completed and the capsule of Bowman is well shown. The tuft of epithelial-like cells, among which endothelial are intermingled, shows as yet no evidence of vascularization. This figure demonstrates clearly the ease with which the terminal walls of the convoluted tubule can be seen and also how these intratubular changes can be distinguished from those taking place outside the tubule. About the tubules many white blood-cells and phagocytes are evident. Vacuolization of cells, exactly as occurred in the formation of the sinuses, is well shown in the lower right quadrant of the figure.

FIG. 7.—Seven days' growth of metanephros of 8-day chick embryo, illustrating the same points as figure 5.

PLATE 3.

FIG. 8.—Five days' growth of metanephros of 9-day chick embryo; 16 mm. lens. Collecting tubules have grown straight out into the marginal growth, carrying with them the undifferentiated nephrogenic tissue which went on to complete differentiation in their final position. They have grown out as intact tubules. The characteristic type of growth for the collecting and convoluted tubules is well shown, the former growing in a straight line and the latter being coiled. At the uppermost point in the figure there is a very small glomerulus in which red blood-cells can be seen. Union of the convoluted and collecting tubules has occurred.

FIG. 9.—Higher power picture of Figure 8; 4 mm. lens, showing extreme upper pole of collecting tubule, convoluted tubule, and glomerulus. The characteristic convolutions of the secreting tubule are well shown, despite the fact that the growth is in the margin where the physical factors attending it were entirely different from those obtaining in the embryo. The glomerulus



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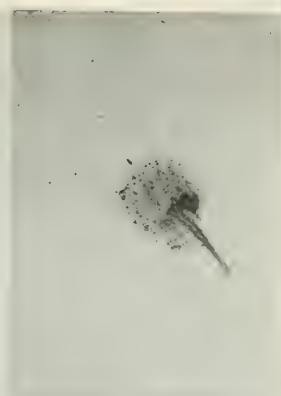
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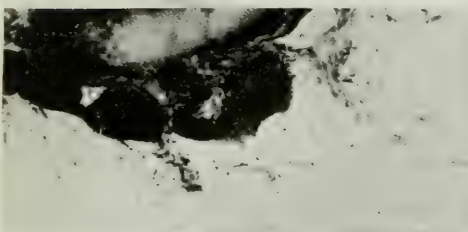
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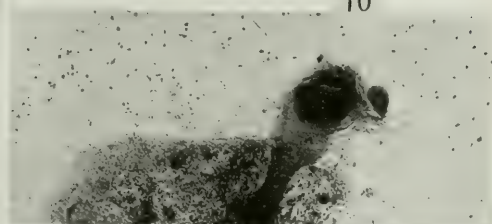
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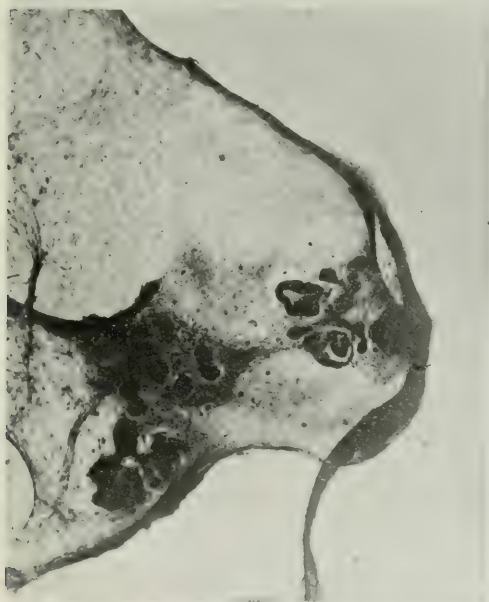
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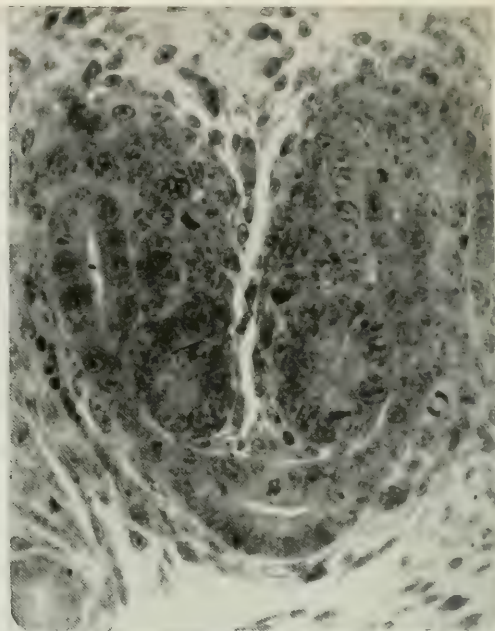
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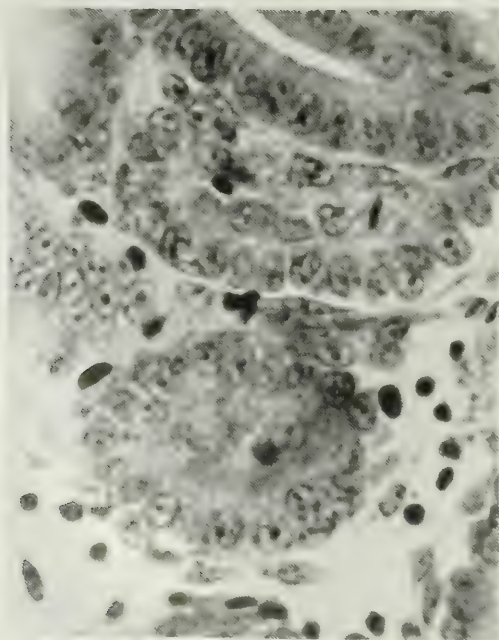
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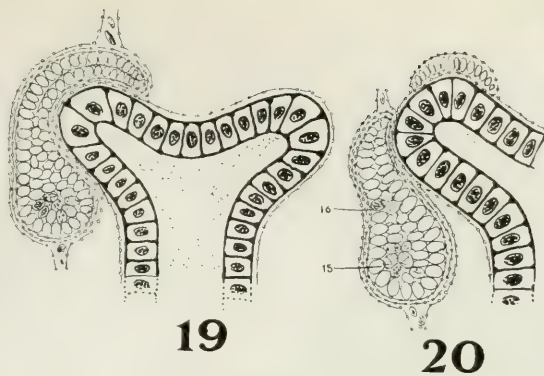
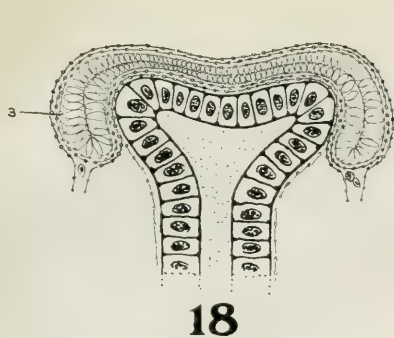
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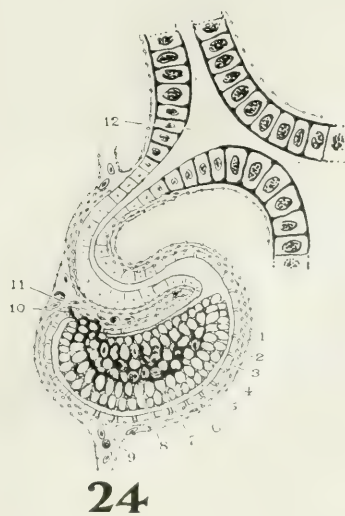
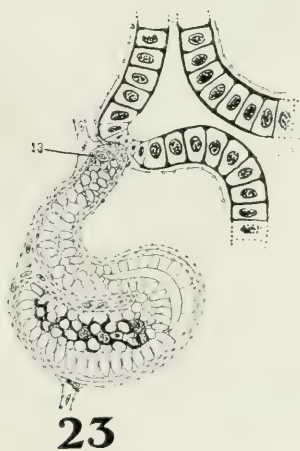
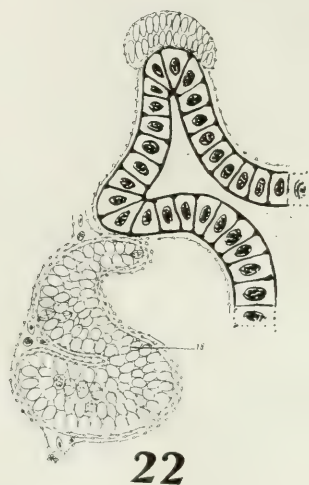
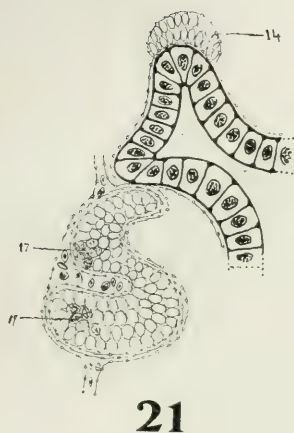
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appears as a small sphere in which red blood-cells are seen as black dots. The defining endothelial cells surrounding the tubules show plainly.

FIG. 10.—4 mm. lens. Three days' growth of metanephros of 9-day chick embryo. Small explant containing collecting tubule, which presents a very interesting growth. The forward or normal growth is accompanied by the usual dichotomous type of branching, while the posterior growth is in a straight line, with no tendency to branch. On either side of the collecting tubule, in the angles, there are two \mathcal{E} -shaped tubules which differentiated in their characteristic position as regards the collecting tubule. These \mathcal{E} -shaped tubules differentiated in the culture. The right side is at a more advanced stage than the left side. Certainly the tendency of the convoluted tubules to be coiled in this culture may be attributed to an inherent tendency of the cells. The collecting tubule, for the same reason, has grown in a straight line.

FIG. 11.—4 mm. lens. Four days' growth of metanephros of 9-day chick embryo. Immediately at the beginning of the marginal outgrowth there are two convoluted tubules with their glomeruli in the course of development. The one to the right side is not as far advanced as the one to the left. Lumen formation has occurred in the tubule to the left. The tubule to the right is a solid core of cells with a dilated bulbous end in which the formation of a lumen has not as yet occurred. The characteristic pinched-off appearance of the neck of the glomerulus, which results from the relatively slow growth of the cells composing this region, is shown.

FIGS. 12 and 13.—Five days' growth of metanephros of 9-day chick embryo. Sinus formation well shown about the convoluted tubules. The white blood-cells show up as dark spots, more pronounced in Figure 12.

FIG. 14.—Same as Figures 12 and 13, except that the sinuses are located more in the denser region about the convoluted tubules.

PLATE 4.

FIG. 15.—Metanephros, 8-day embryo. Section 5 micra. $\times 750$. Collecting tubule formation with inverted comma-shaped mass of nephrogenic tissue about end. The cells making up the body and tail of comma are spherical in type with their axes perpendicular to the central core. In the head of the comma there is marked proliferation evidenced by mitotic figures. About the periphery of the comma-shaped mass the endothelial cells can be seen with their axes parallel to the central core. These endothelial cells are just in a stage of transformation from the round spherical type to the long slender endothelial type. The head of the comma is turned somewhat towards the observer and at the inner angle about the middle of the mass (at X) is an endothelium-lined space which approximates the sphere and, when several sections are studied, proves to communicate with an endothelium-lined sinus, which is somewhat poorly shown in this figure, just beneath the head of the comma. This is the portion of the sinus surrounding the comma-shaped mass that will later be included in the first curve of the \mathcal{E} -shaped tubule. This figure shows the comma just prior to the development of the lumen and demonstrates very clearly that the tail and body of the comma, as well as the head, are utilized in the formation of the \mathcal{E} -shaped tubule.

FIG. 16.—Metanephros, 9-day embryo. Section 5 micra. $\times 750$. A "Y"-shaped collecting tubule with two comma-shaped bodies along its inner sides and about its ends. Between the commas there is an endothelium-lined sinus which surrounds the entire anlage. The endothelial walls of the sinus are in opposition about the lateral side of each of the collecting tubules, but the connection with the large sinus at the lower portion of the

figure can be traced even in the photograph. The tubule is here shown in a later stage of development than in Fig. 1. The endothelial covering is evident, as well as the endothelium lining the sinus, the two layers being in apposition. Lumen formation has just begun and is somewhat more advanced on the right side than on the left. The characteristic polarity of the endothelial and epithelial cells, as well as the more rapid proliferation of the spherical head pole of the tubule, is well shown.

FIG. 17.—Metanephros, 9-day embryo. Section 5 micra. $\times 1500$. Cellular tuft in glomerular end of convoluted tubule shown immediately below a loop of the tubule. The lower loop of tubule belongs to another unit. The capsule of Bowman is partially formed about the spherical tuft of cells and the tuft is bounded by a row of radially arranged epithelial-like cells. The flattened epithelial cells making up the parietal layer of Bowman's capsule are evident. The point of junction of the glomerular end with the convoluted tubule occurs just to the right in the figure. A double line of flattened endothelial cells running across the superior surface of the glomerular tuft is evident. In the distal end of the tuft a black spot is seen which is a newly formed red blood-cell. On each side of this cell there are endothelial cells which form a channel in the middle of the epithelial cells making up the major portion of the tuft. This is better shown in Plate 5. A mitotic figure can be seen towards the proximal end of the tuft. The clear areas in the figure, in which red blood-cells appear as black spots, are the sinuses in the immediate neighborhood of the glomerulus.

PLATE 5.

FIG. 18.—Collecting tubule covered by cap of metanephrogenic tissue. The collecting tubule contains granules and is lined with columnar epithelium. Three layers of endothelium can be seen about the cap and one layer surrounding the collecting tubule. The spherical epithelial cells composing the cap are radially arranged about a central core corresponding to the single line shown. Blood cells are shown in the sinus existing between the two outermost endothelial layers.

FIG. 19.—Collecting tubule buds increased in length. The metanephrogenic cap has become transformed into an inverted comma-shaped mass. The cells making up the tail of the comma show the same arrangement as in Fig. 18. The head of the comma is the seat of most marked proliferation causing a dilatation of the head pole.

FIG. 20.—Collecting tubule only partly shown. Growth of bud extending peripheralward carrying small portion of metanephrogenic tissue. The comma-shaped mass has approximated its definitive position in regard to the collecting tubule. At points 15 and 16 the most rapid proliferation of cells takes place.

FIG. 21.—Transition of comma-shaped mass into that of an \mathcal{E} -shaped tubule has occurred, angulation having been brought about by proliferation and more rapid growth of cells at points 17 and 19, point 17 being the first curve of the \mathcal{E} -shaped tubule formed. A sac-like protrusion of the sinus (No. 11 in Fig. 24) has been included between the superior surface of the bulbous glomerular end (19) and the inferior surface of the first curve (17). Blood cells are shown in this sac. Metanephrogenic cap shown at 14.

FIG. 22.—Collecting tubule and metanephrogenic cap remain about the same as in Fig. 21. \mathcal{E} -shaped tubule more angulated. Second curve No. 18. First curve increased in size. Glomerular end a dilated bulb of spherical epithelial cells.

FIG. 23.— \mathcal{E} -shaped tubule growing in the collecting tubule at point 13. Lumen formation occurring in second curve with abutting cells becoming cuboidal in shape. Heavy black lines in tuft of epithelial cells composing glomerular end are the endothelial cells, No. 8 in Fig. 24.

FIG. 24.—Z-shaped tubule completely joined with collecting tubule. Lumen complete with the formation of Bowman's capsule (5). Note that this formation is entirely inside the endothelial covering layers 1, 2 and 3, 4 constituting the parietal layer of Bowman's capsule and 6 the visceral; 7, spherical epithelial cells composing the tuft surrounded by Bowman's capsule. Inter-twining endothelial cells and channels (8) among the epithelial

cells. Blood elements are also shown forming *in situ*. The connection of the endothelium inside the tuft with the surrounding sinus is shown at 10, and the included endothelial sac or sinus at 11; 9, a connection between the surrounding endothelial sinus and sinuses in other parts of the metanephros. The lumen of the collecting tubule is shown at 12.

BIOMETRICAL STUDIES IN PATHOLOGY. II

PATHOMETRIC INDEX NUMBERS:

PRELIMINARY ACCOUNT OF A QUANTITATIVE METHOD OF EVALUATING PATHOLOGICAL PHENOMENA¹

By RAYMOND PEARL²

THE PROBLEM

An autopsy protocol is essentially and in the main a qualitative description of the state of the organs of the body at death. Its fundamental purpose is to make possible in some degree the reconstruction of the course of physiological (or pathological) events which led to death, so that a correlation may be effected between clinical findings and the actual state of the organs of the body. That this is a most important and useful purpose, which in proportion as it is fulfilled aids enormously the progress of scientific medicine, no thoughtful person will deny.

But it is possible to conceive a still broader and more fundamental usefulness of pathological anatomy even than this which has been sketched, namely, as an aid in the solution of problems of general biology. Pathological phenomena are biological phenomena. The decay and breakdown of organs is just as much and just as truly an essential part of the whole normal life history of an organism as are, for example, growth phenomena. That pathological anatomy is not usually thought of by biologists as an intrinsic part of the subject matter of their science is really only the expression of an accident of specialization in human activity. But because of the fact that, in the main, pathology has been developed primarily for its strictly medical usefulness, it has resulted that its broader biological aspects have been, if not neglected, at least not developed to the extent that seems possible. There are almost innumerable biological problems presented by the data of pathology which have not been touched even. A certain restricted group of these problems has interested me greatly, and the present paper is offered as a first attempt, subject to elaboration and revision with further study, at an attack upon them.

If one reads over carefully a great number of autopsy protocols something like the following thought forces itself upon his mind. There is a great deal of variation in the amount and extent of observable pathological lesions

associated with death. In some cases one sees enormous alterations of structure and function affecting nearly or quite all the important organs of the body. In other cases all that can be found at autopsy are relatively slight, or even apparently insignificant lesions of a few organs. Between these two extremes are all degrees of variation in the pathological picture. The whole situation, looked at in a naive way, is as though it required a great deal more disturbance of structure and function to kill some people than it does to kill others. In other words, while the whole pathological picture, as presented in the protocol, is essentially a qualitative one, nevertheless as soon as one starts to think about it, he wants to think in quantitative terms.

Such thinking at once leads to some further considerations. There is suggested a concept, which for lack of a better term, may be called that of the *specific vital resistance* of an individual. If individual *A* lives along for a considerable period of time with a number of extremely grave organ lesions, while individual *B* dies promptly upon the appearance of a few relatively slight disturbances of structure and function, we may conceive that *A* has a higher specific vital resistance to death than *B*. It takes more to kill *A* than it does *B*. If we can get some sort of reliable quantitative measure of what we have called specific vital resistance it will at once open out many lines of research. How do the different races and the two sexes, for example, compare in respect of this presumably fundamental biological quality?

The converse of the concept of specific vital resistance is that of *specific pathopoiesis*,³ the tendency of the individual to become pathological, or to develop alterations

¹ Papers from the Statistical Department of the Johns Hopkins Hospital. No. 4.

² Assisted by Agnes Latimer Bacon.

³ The use of this term quite obviously puts much weight upon the part played by the individual, *qua* individual, in his own becoming diseased. This is deliberate, and in accord with the writer's general view, that biologically the innate constitution of the host organism is at least as important a factor, broadly speaking, in the etiology of disease, as is the invading organism, even in most infectious diseases, and probably a much more important factor than any or all external agents in the etiology of the so-called "organic" diseases.

of structure and function from the normal. According to this concept the individual, who, upon autopsy, exhibits many and grave lesions is more highly pathopoietic than another who shows only slight and insignificant lesions. Is the negro, in this sense, more highly pathopoietic than the white?

There are many angles of approach and possibilities inherent in this general idea which has been sketched. Will a proper quantitative measure of the general autopsy picture tend to have higher or lower values in those dying, for example, primarily because of disturbances in the functioning of the heart, than in those dying primarily because of disturbances in the functioning of the liver? And similarly for other organs.

I shall not attempt in this preliminary paper to outline the problems which can be attacked if a proper method of quantitatively evaluating a general pathological picture can be devised. Instead I shall leave this to the imagination and insight of the reader, and proceed to state the method by which I have approached the problem of setting up such a measure.

A SUGGESTED PATHOMETRIC INDEX NUMBER

In developing a pathometric index number I have followed the same idea that was developed in my laboratory [Pearl and Surface (1909), and Pearl (1912)] some years ago in a series of papers, in connection with the study of certain problems of genetics. It was there pointed out that:

"An 'index number' in the sense here used means a single mathematical function which combines in itself the values of several independent or correlated variables. In such a function each of the variables may be weighted in any desired manner to meet the needs of the particular problem. Having decided the relative degree to which each variable shall be weighted, the index number as finally calculated gives an absolutely impartial and impersonal summing up of the total combined value or effect of the variables entering into it.

"The theory of a breeding index number may be illustrated by a concrete example. Let the matter of improving maize by selective breeding be taken. Suppose that a breeder starts with a promising variety of yellow dent corn. This variety, while promising, has never been improved by breeding at all. The ears are only fair in respect to size and shape. The principal aim of the breeder of this corn, let us suppose, is to increase the 'earliness' (i. e., shorten the time of maturing), but at the same time he wishes to improve the other characteristics of the corn—size and shape of ear, relative proportion of corn on cob, and yield per acre. In making field selections of plants to furnish seed for testing out by the 'ear-row' or other method, the breeder will endeavor to select with reference to as many as possible of the points enumerated above in addition to 'earliness.' Further it will be desired after the ears have been harvested and dried to take careful account in selecting the seed of at least the following points: Shape of ear, length of ear, circumference of ear, condition of tip and butt of the ear, kernel shape, germination and proportion of shelled corn to cob. Now if a plant happens to be specially early, even though it be relatively poor in respect to these ear characters, it is likely to be selected to furnish seed, in spite of these defects. But it is possible to devise a formula for a selection index number which shall give whatever weight may be

previously agreed upon to each of these several variable characters of the corn which have been enumerated. Having settled upon a particular formula, the selection of seed then becomes essentially a purely mechanical matter so far as the characters included in the formula are concerned. The value of the index will be determined by the relative contributions from each of the included variables. If the breeder calculates such a selection index number, and takes no ears with an index below that standard, it will then be possible for him to select with reference to a series of characters in an unbiased and impartial manner."

The quantification of the whole pathological picture presented by an autopsy protocol offers a closely similar problem to that dealt with in forming breeding index numbers.

There are certainly four elements as factors in the case which must be considered in making up a pathometric index number. There are:

1. *The magnitude and gravity of the lesion, as lesion.*—A single small apical scar in the lung is a slight and insignificant lesion of tuberculosis, as compared with a large ulcerating cavity resulting from the presence in the lungs of tubercle bacilli. The comparative extent and gravity of any given lesion, as compared with other lesions of the same sort, may be indicated by a numerical weighting on a scale of 1 to 10, 1 denoting the smallest and most significant lesion of the sort dealt with, and 10 the largest and most grave ever found. The assignment of the weights will depend, of course, upon the careful study of the description in the protocol, and upon the judgment and knowledge of the worker. If the *same* individual does all the weighting, however, on a given series of autopsies, the results will be comparable *inter se*.

2. *The organ or organ system in which the lesion occurs.*—It is obvious *a priori* that any lesion, however insignificant or grave, is more important pathologically, i. e., to life or death, if it occurs in some organs than if it occurs in some others, because some organs are fundamentally more important to the general physiological economy than others. Thus, for example, a lesion of the skin, say an epithelioma, is much less likely *per se* to cause death, than a carcinoma of the liver. How shall this idea be expressed quantitatively? In general terms the answer is obvious; we should give numerical weights to the several organ systems in proportion to their importance in the general physiological economy. After careful thought about the matter, it has seemed that the best *available* indication of the relative importance of the several organ systems, is to be found in the relative frequency with which their breakdown (structural and functional) leads to death. In an earlier study (Pearl, 1920) I have allocated the death rates from the several statistically recognized causes of death back to the organ systems, the breakdown of which is primarily responsible for the lethal effect of that cause. As a first approximation to organ weightings I have taken from that paper (which should be read in connection with the present study) the following death-rates as weighting figures.

<i>Organ System</i>	<i>Weight</i>
Respiratory system.....	396
Alimentary canal, and associated organs of metabolism	335
Circulatory system, blood and blood forming organs	210
Nervous system and sense organs.....	176
Kidneys and related excretory organs.....	107
Primary and secondary sex organs.....	88
Skeletal and muscular system.....	13
Skin	10
Endocrinal system.....	2
"Body as a whole".....	1507

These weights represent the proportion in which breakdown of the indicated organ systems actually do cause death in the United States Registration Area, so far as may be judged from certified causes of death.

The rubric "Body as a whole" with a weight which is the sum of the separate organ weights, is included to care for pathological conditions not referable, on the basis of any present knowledge, to any particular organ system, to the exclusion of others but which involve the whole body as a functioning machine. Such cases are mainly profound disturbances of metabolism, such as, for example, rickets.

3. *The etiological factor involved in the production of the lesion.*—The influences upon the chances of life or death of the individual vary greatly, with lesions of the same extent in the same organ, according to the etiological factor behind the lesion. For example, a traumatic lesion of the skin, as from a burn, is a very different thing in respect of prognosis than an equally extensive lupus lesion. As a first attempt at the quantification of this idea the following system of etiological weights has been set up:

<i>Etiological Factor</i>	<i>Weight</i>
Malignant neoplasm.....	10
Infection	8
Poison (inorganic or organic).....	6
Trophic or organic functional disturbance.....	5
Trauma	3
Structural (inherited or congenital) abnormality	2
Benign neoplasm.....	1
Non-bacterial parasites (e.g. worms).....	1

The assignment of these weights is entirely arbitrary but tries in a general way to base itself upon the prognosis in the presence of these various etiological factors.

4. *The age of the person.* A lesion of given magnitude, in a particular organ system, and of a given etiology, has a different pathological significance if present in a baby, to what it has if present in a man of 70 years, let us say. In general, the age of the individual must surely have a place in any quantification of the pathological picture. In the pathometric index about to be described it has been decided, after trial of several other plans, to introduce the age factor in this form of the actuarial constant $\overset{\circ}{e}_x$, which is the complete expectation of life at a given age.

How now shall these four essential elements in the problem be combined to form a single index number? After careful consideration and experimentation the following function has been chosen.

$$I_p = \frac{\sum \{ (\overset{\circ}{V}b^a) \}^c}{1000} \times \overset{\circ}{e}_x$$

In this expression

I_p = pathometric index number for a particular autopsy protocol.

Σ = summation to include each and all lesions present in the case.

b = organ system weight.

a = lesion weight.

c = etiological weight.

$\overset{\circ}{e}_x$ = complete expectation of life at attained age of individual, from life table.

The function

$$(\overset{\circ}{V}b^a)^c$$

can obviously be tabled once for all for the 10 assigned weights of b , and values of a and c ranging from 1 to 10 inclusive. Such tables have been calculated and are here given as Tables 1 to 10.

TABLES OF FUNCTION $(\overset{\circ}{V}b^a)^c$ FOR ASSIGNED VALUES OF b .

TABLE 1. $b = 2$.

	a										
c	1	2	3	4	5	6	7	8	9	10	
1	1.15	1.32	1.52	1.74	2.00	2.30	2.64	3.03	3.48	4.00	
2	2.30	2.64	3.03	3.48	4.00	4.59	5.28	6.06	6.96	8.00	
3	3.45	3.96	4.55	5.22	6.00	6.89	7.92	9.09	10.45	12.00	
4	4.59	5.28	6.06	6.96	8.00	9.19	10.56	12.13	13.93	16.00	
5	5.74	6.60	7.58	8.71	10.00	11.49	13.19	15.16	17.41	20.00	
6	6.89	7.92	9.09	10.45	12.00	13.78	15.83	18.19	20.89	24.00	
7	8.04	9.24	10.61	12.19	14.00	16.08	18.47	21.22	24.38	28.00	
8	9.19	10.56	12.13	13.93	16.00	18.38	21.11	24.25	27.86	32.00	
9	10.34	11.88	13.64	15.67	18.00	20.68	23.75	27.28	31.34	36.00	
10	11.49	13.19	15.16	17.41	20.00	22.97	26.39	30.31	34.82	40.00	

TABLE 2. $b = 10$.

c	a	1	2	3	4	5	6	7	8	9	10
1	1.58	2.51	3.98	6.31	10.00	15.85	25.12	39.81	63.10	100.00	
2	3.17	5.02	7.96	12.62	20.00	31.70	50.24	79.62	126.19	200.00	
3	4.75	7.54	11.94	18.93	30.00	47.55	75.36	119.43	189.29	300.00	
4	6.34	10.05	15.92	25.24	40.00	63.40	100.48	159.24	252.38	400.00	
5	7.92	12.56	19.91	31.55	50.00	79.24	125.59	199.05	315.48	500.00	
6	9.51	15.07	23.89	37.86	60.00	95.09	150.71	238.86	378.57	600.00	
7	11.09	17.58	27.87	44.17	70.00	110.94	175.83	278.68	441.67	700.00	
8	12.68	20.10	31.85	50.48	80.00	126.79	200.95	318.49	504.77	800.00	
9	14.26	22.61	35.83	56.79	90.00	142.64	226.07	358.30	567.86	900.00	
10	15.85	25.12	39.81	63.10	100.00	158.49	251.19	398.11	630.96	1000.00	

TABLE 3. $b = 13$.

c	a	1	2	3	4	5	6	7	8	9	10
1	1.67	2.79	4.66	7.78	13.00	21.71	36.27	60.58	101.18	169.00	
2	3.34	5.58	9.32	15.57	26.00	43.43	72.54	121.15	202.36	338.00	
3	5.01	8.37	13.98	23.35	39.00	65.14	108.80	181.73	303.54	507.00	
4	6.68	11.16	18.64	31.13	52.00	86.85	145.07	242.31	404.72	676.00	
5	8.35	13.95	23.30	38.92	65.00	108.57	181.34	302.89	505.90	845.00	
6	10.02	16.74	27.96	46.70	78.00	130.28	217.61	363.46	607.08	1014.00	
7	11.69	19.53	32.62	54.48	91.00	152.00	253.87	424.04	708.26	1183.00	
8	13.36	22.32	37.28	62.27	104.00	173.71	290.14	484.62	809.45	1352.00	
9	15.03	25.11	41.94	70.05	117.00	195.42	326.41	545.19	910.63	1521.00	
10	16.70	27.90	46.60	77.83	130.00	217.14	362.68	605.77	1011.81	1690.00	

TABLE 4. $b = 88$.

c	a	1	2	3	4	5	6	7	8	9	10
1	2.45	6.00	14.68	35.94	88.00	215.47	527.56	1291.73	3162.78	7744.00	
2	4.90	11.99	29.36	71.88	176.00	430.93	1055.13	2583.46	6325.56	15488.00	
3	7.35	17.99	44.03	107.82	264.00	646.40	1582.69	3875.19	9488.34	23232.00	
4	9.79	23.98	58.71	143.76	352.00	861.86	2110.26	5166.93	12651.12	30976.00	
5	12.24	29.98	73.39	179.70	440.00	1077.33	2637.82	6458.66	15813.90	38720.00	
6	14.69	35.97	88.07	215.64	528.00	1292.80	3165.39	7750.39	18976.68	46464.00	
7	17.14	41.97	102.75	251.58	616.00	1508.26	3692.95	9042.12	22139.46	54208.00	
8	19.59	47.96	117.42	287.53	704.00	1723.73	4220.52	10333.85	25302.23	61952.00	
9	22.04	53.96	132.10	323.47	792.00	1939.20	4748.08	11625.58	28465.01	69696.00	
10	24.48	59.95	146.78	359.41	880.00	2154.66	5275.65	12917.32	31627.79	77440.00	

TABLE 5. $b = 107$.

c	a	1	2	3	4	5	6	7	8	9	10
1	2.55	6.48	16.51	42.02	107.00	272.43	693.65	1766.09	4496.07	11449.00	
2	5.09	12.97	33.01	84.05	214.00	544.87	1387.29	3532.19	8993.34	22898.00	
3	7.64	19.45	49.52	126.07	321.00	817.30	2080.94	5298.29	13490.00	34347.00	
4	10.18	25.93	66.02	168.10	428.00	1089.73	2774.58	7064.38	17986.67	45796.00	
5	12.73	32.41	82.53	210.12	535.00	1362.17	3468.23	8830.47	22483.34	57245.00	
6	15.28	38.90	99.03	252.15	642.00	1634.60	4161.87	10596.57	26980.01	68694.00	
7	17.82	45.38	115.54	294.17	749.00	1907.03	4855.52	12362.67	31476.68	80143.00	
8	20.37	51.86	132.04	336.20	856.00	2179.47	5549.16	14128.76	35973.34	91592.00	
9	22.91	58.34	148.55	378.22	963.00	2451.90	6242.81	15894.85	40470.01	102041.00	
10	25.46	64.83	165.06	420.25	1070.00	2724.34	6936.45	17660.95	44966.68	114490.00	

TABLE 6. $b = 176$.

c	a	1	2	3	4	5	6	7	8	9	10
1	2.81	7.91	22.25	62.58	176.00	495.01	1392.22	3915.80	11013.44	30976.00	
2	5.63	15.82	44.50	125.15	352.00	990.02	2784.44	7831.60	22026.87	61952.00	
3	8.44	23.73	66.75	187.73	528.00	1485.03	4176.66	11747.40	33040.31	92928.00	
4	11.25	31.64	89.00	250.31	704.00	1980.05	5568.88	15663.19	44053.75	123904.00	
5	14.06	39.55	111.24	312.88	880.00	2475.06	6961.10	19578.99	55067.19	154880.00	
6	16.88	47.46	133.49	375.46	1056.00	2970.07	8353.32	23494.79	66080.62	185856.00	
7	19.69	55.37	155.74	438.03	1232.00	3465.08	9745.54	27410.59	77094.06	216832.00	
8	22.50	63.28	177.99	500.61	1408.00	3960.09	11137.76	31326.39	88107.50	247808.00	
9	25.31	71.19	200.24	563.19	1584.00	4455.10	12529.98	35242.19	99120.93	278784.00	
10	28.13	79.11	222.49	625.76	1760.00	4950.11	13922.20	39157.98	110134.37	309760.00	

TABLE 7. $b = 210$.

c	a	1	2	3	4	5	6	7	8	9	10
1	2.91	8.49	24.74	72.07	210.00	611.88	1782.82	5194.59	15135.43	44100.00	
2	5.83	16.98	49.47	144.15	420.00	1223.75	3565.64	10389.17	30270.86	88200.00	
3	8.74	25.47	74.21	216.22	630.00	1835.63	5348.45	15583.76	45406.29	132300.00	
4	11.65	33.96	98.94	288.29	840.00	2447.50	7131.27	20778.34	60541.72	176400.00	
5	14.57	42.45	123.68	360.37	1050.00	3059.38	8914.09	25972.93	75677.15	220500.00	
6	17.48	50.94	148.42	432.44	1260.00	3671.25	10696.91	31167.51	90812.58	264600.00	
7	20.40	59.43	173.15	504.51	1470.00	4283.13	12479.73	36362.10	105948.01	308700.00	
8	23.31	67.92	197.89	576.59	1680.00	4895.00	14262.54	41556.68	121083.44	352800.00	
9	26.22	76.41	222.63	648.66	1890.00	5506.88	16045.36	46751.27	136218.87	396900.00	
10	29.14	84.90	247.36	720.73	2100.00	6118.76	17828.18	51945.85	151354.30	441000.00	

TABLE 8. $b = 335$.

c	a	1	2	3	4	5	6	7	8	9	10
1	3.20	10.23	32.74	104.72	335.00	1071.65	3428.17	10966.59	35081.70	112225.00	
2	6.40	20.47	65.47	209.44	670.00	2143.30	6856.35	21933.19	70163.40	224450.00	
3	9.60	30.70	98.21	314.16	1005.00	3214.96	10284.52	32899.78	105245.11	336675.00	
4	12.80	40.93	130.94	418.89	1340.00	4286.61	13712.69	43866.37	140326.81	448900.00	
5	15.99	51.17	163.61	523.61	1675.00	5358.26	17140.86	54832.97	175408.51	561125.00	
6	19.19	61.40	196.42	628.33	2010.00	6429.91	20569.04	65799.56	210490.21	673350.00	
7	22.39	71.63	229.15	733.05	2345.00	7501.56	23997.21	76766.15	245571.91	785575.00	
8	25.59	81.87	261.89	837.77	2680.00	8573.22	27425.38	87732.74	280653.62	897800.00	
9	28.79	92.10	294.62	942.49	3015.00	9644.87	30853.55	98699.34	315735.32	1010025.00	
10	31.99	102.33	327.36	1047.22	3350.00	10716.52	34281.73	109665.93	350817.02	1122250.00	

TABLE 9. $b = 396$.

c	a	1	2	3	4	5	6	7	8	9	10
1	3.31	10.94	36.19	119.72	396.00	1309.89	4332.84	14332.18	47407.97	156816.00	
2	6.62	21.88	72.38	239.43	792.00	2619.78	8665.67	28664.36	94815.95	313632.00	
3	9.92	32.82	108.58	359.15	1188.00	3929.66	12998.51	42996.55	142223.92	470448.00	
4	13.23	43.77	144.77	478.87	1584.00	5239.55	17331.35	57328.73	189631.90	627264.00	
5	16.54	54.71	180.96	598.59	1980.00	6549.44	21664.18	71660.91	237029.87	784080.00	
6	19.85	65.65	217.15	718.30	2376.00	7859.33	25997.02	85993.09	284447.84	940896.00	
7	23.15	76.59	253.35	838.02	2772.00	9169.22	30329.85	100325.27	331855.82	1097712.00	
8	26.46	87.53	289.54	957.74	3168.00	10479.11	34662.69	114657.46	379263.79	1254528.00	
9	29.77	98.47	325.73	1077.45	3564.00	11788.99	38995.53	128989.64	426671.77	1411344.00	
10	33.08	109.42	361.92	1197.17	3960.00	13098.88	43328.36	143321.82	474079.74	1568160.00	

TABLE 10. $b = 1507$.

c	a	1	2	3	4	5	6	7	8	9	10
1	4.32	18.67	80.70	348.73	1507.00	6512.32	28142.24	121613.36	525537.26	2271049.00	
2	8.64	37.35	161.40	697.46	3014.00	13024.65	56284.47	243226.72	1051075.52	4542098.00	
3	12.96	56.02	242.10	1046.19	4521.00	19536.97	84426.71	364840.08	1576613.28	6818147.00	
4	17.29	74.70	322.80	1394.92	6028.00	26049.29	112568.94	486453.44	2102151.04	9084196.00	
5	21.61	93.37	403.49	1743.66	7535.00	32561.62	140711.18	608066.80	2627688.80	11355245.00	
6	25.93	112.05	484.19	2092.39	9042.00	39073.94	168853.41	729680.16	3153226.56	13626294.00	
7	30.25	130.72	564.89	2441.12	10549.00	45586.26	196995.65	851293.52	3678764.32	15897343.00	
8	34.57	149.39	645.59	2789.85	12056.00	52098.59	225137.88	972906.88	4204302.08	18168392.00	
9	38.89	168.07	726.29	3138.58	13563.00	58610.91	253280.12	1094520.24	4729839.84	20439441.00	
10	43.21	186.74	806.99	3487.31	15070.00	65123.23	281422.35	1216133.60	5255377.60	22710490.00	

It is now desirable to illustrate the application of this formula by going through the steps of calculating the pathometric index from a particular protocol. The following may be taken as a typical illustrative case.

Autopsy No. 5030. White female. *act.* 33.

Anatomical diagnosis.

Primary: Syphilitic mesoarteritis; aneurysm of ascending arch of aorta; erosion of second and third ribs, right; compression of pulmonary veins; hydrothorax, right; infarcts of kidney and heart; rupture of aneurysm into pericardium.

Subsidiary: Fibrous pleurisy; encapsulated caseous and calcified nodules in the spleen and left lung.

We then set up the following system of weights:

Organ	Lesion Weight A	Organ Weight B	Etiological Weight C	(From Tables)
Aorta	10	210	8	352800.00
Ribs	6	13	8	173.71
Lungs and pleura	5	396	8	3168.00
Heart	6	210	8	4895.00
Kidneys	3	107	8	132.04
Spleen	2	210	8	67.92
			$\Sigma =$	361236.67
			$\Sigma \div 1000 =$	361.24

Studying the protocol carefully leads to the assignment of the lesion weights in column A. The organ weights are from the table given above. The etiological factor behind all these lesions is infection, either syphilitic or tuberculous.

From Grover's (1921) life tables we find on p. 110 that for white females living in cities in the Registration Area, aged 33-34

$$\bar{e}_x = 33.20$$

Finally, multiplying 361.24 by 33.20, we get

$$I_p = 11993.$$

It will be seen, in the first place, that with the aid of the tables given above the arithmetical work is reduced to a minimum. By examining the detailed figures it appears that quantitatively the aneurysm accounts for the major portion of the final value of the index, as it ought to, and the contributions of the other lesions to the final

result are in about the proportions that common sense would assign them. We have reached a quantitative evaluation of the pathological picture presented by this autopsy.

THE DISTRIBUTION OF THE PATHOMETRIC INDEX

In order to give some indication of the range of values which this index number I_p may take in actual practice, its value was worked out for 200 consecutive autopsies beginning with autopsy No. 5000. In this series each successive protocol was taken as it came, except that in general all autopsies of still-born infants, and those dying within a few days of birth were omitted, in order that so short a series as 200 might not be unduly weighted with cases of this sort. Also, of course, all incomplete autopsies (mostly brain only) were omitted.

The values of the index for these 200 cases as shown in Table 11, which is arranged in the form of a correlation table with duration of present illness as the second variable. Duration of present illness was extracted from the case histories as the second variable, as an example of general interest in connection with the underlying idea of pathometric index numbers. Is there a significant correlation, when quantitatively measured, between the length of time a person has been ill (*i. e.*, has had clinical symptoms recognizable by himself as existing) and the amount of pathological disturbance found at autopsy?

In this group of 200 cases the index takes values all the way from nearly zero to over 100,000. Over one-half the cases however fall below 5000 in the value of the index. The distribution is plainly extremely skew in the positive direction. A matter of great interest for future work will be to determine the amount and character of variation in the index for groups homogeneous in respect of age. A considerable part of the skewness of the index distribution here shown is undoubtedly due to the fact that in 62 of the 200 autopsies the individual was under 5 years of age.

The biometric constants (which have little meaning because of the deliberately non-homogeneous character of the material in respect of age, race, sex, etc.) from Table 11 are exhibited in Table 12.

TABLE 11.
Values of the pathometric index I_p for 200 autopsies.

	0-4999	5000-9999	10000-14999	15000-19999	20000-24999	25000-29999	30000-34999	35000-39999	40000-44999	45000-49999	50000-54999	55000-59999	60000-64999	65000-69999	70000-74999	75000-79999	80000-84999	85000-89999	90000-94999	95000-99999	100000-104999	105000-109999	Total
Under 1 wk.	15	3	—	1	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	21
1-3.9 wks.	27	8	8	4	5	—	2	—	—	1	—	—	—	—	—	—	—	—	—	—	2	—	57
1-5.9 mos.	21	9	9	3	2	3	1	1	1	—	—	—	—	—	—	—	—	—	—	1	—	—	51
6-11.9 mos.	12	3	1	1	—	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1	22
1-1.9 yrs.	6	3	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	11
2-2.9 yrs.	7	—	2	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	11
3-3.9 yrs.	8	1	4	—	1	1	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	17
4-4.9 yrs.	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
5-5.9 yrs.	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
6-6.9 yrs.	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
7-7.9 yrs.	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	104	27	27	11	8	7	5	1	3	3	—	—	—	—	—	—	—	—	—	1	2	1	200

TABLE 12.
Biometric constants from Table 11.

Variable	Mean	Standard deviation	Coefficient of variation
Pathometric index..	11,225 \pm 790	16,560 \pm 558	148 \pm 12
Dur. present illness	355.2 \pm 26.5 d.	555.7 \pm 18.7 d.	156 \pm 13

Coefficient of correlation = $-.071 \pm .047$.

The constants of variation have high values, presumably in large part due to the non-homogeneity of the material. The correlation between pathometric index and duration of illness is sensibly zero, for this particular sample of material. It may well be that with more homogeneous material a definite correlation would emerge. This will be tested in future work.

SUMMARY

In this paper is presented a preliminary account of a pathometric index number, which has for its purpose the

obtaining of a single numerical expression of the gravity of the whole pathological picture disclosed by the lesions discovered at autopsy. The method of calculation is illustrated and the distribution of values of the index for 200 autopsies shown. The purpose of the present paper is purely methodological. Later it is hoped to apply the method to the solution of specific problems.

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VASO-MOTOR REACTIONS *

By W. M. BAYLISS, M. A., D. Sc., F. R. S.

I propose to devote this lecture to a brief consideration of certain recent work, partly my own, on some aspects of the regulation of the circulation. In the first place, the general nature of vascular reflexes requires reference.

To obtain a clear conception of the phenomena, certain general properties of smooth muscle and its innervation must be kept in mind. Like this tissue in other places, the arterial muscle tends naturally when left to itself to be in a state of moderate contraction or "tone." There are various phenomena which demonstrate this fact, but perhaps the best known is that the blood vessels of an organ, after the nerves are cut, can be made either to relax or contract by stimulation of the peripheral ends of

certain nerves supplying the organ. Thus, the arterial muscle is supplied with excitatory and with inhibitory nerves. We call the former, vaso-constrictors, the latter, vaso-dilators. It appears that the former always come from the sympathetic system; the latter from various other sources, but leaving the nervous system in cerebral and spinal nerves. There is usually a certain degree of constant activity of the vaso-constrictors, by impulses from the nerve-centres, probably of reflex origin. The vaso-dilator nerves are only in tonic action under special conditions, those of the skin, for example, under hot and moist states of the atmosphere. The latter appear also to be active in the depressor reflex excited by high blood pressure or an over-full state of the vascular system. This reflex fall of blood pressure brought about by stimulation of the central end of the depressor nerve is familiar

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to all and we may take it as the vascular reflex most completely worked out.

In theory, a fall of blood pressure due to vaso-dilatation, can be obtained in either of two ways—by inhibition of the vaso-constrictor centre when it is in activity, or by excitation of the vaso-dilator centre. Ludwig and Cyon, the discoverer of the depressor nerve, ascribed the effect to the former; Heidenhain and Ostroumov to the latter. The difference of opinion may perhaps be due to the former observers having directed their attention chiefly to the visceral circulation, the innervation of which is preponderantly vaso-constrictor, while the latter observed chiefly the skin, whose innervation is mainly vaso-dilator. In some experiments which I made several years ago, the impression was given me that both components mentioned played a part throughout. This suggestion was made before the famous work of Sherrington on reciprocal innervation in reflexes to voluntary muscles. Since my earlier experiments had shown the probability of a kind of reciprocal innervation in vascular reflexes, I took up the question again, especially as Sherrington had found that strychnine reversed the inhibitory effect of the depressor nerve, as it did the inhibitory component of reflexes to voluntary muscles, and had suggested that I should work it out further.

Taking first the depressor reflex, I found that not only was there an inhibition of the constrictor centre, but also an excitation of the dilator centre. The first was shown by vaso-dilatation in regions from which the vaso-dilator innervation had been cut off, as the hind leg after removal of the lumbar cord; the second, by the presence of dilatation in regions from which the constrictor innervation had been removed, as the submaxillary gland after section of the sympathetic supply. Conversely, in the pressor reflexes from ordinary sensory nerves, there is not only an excitation of vaso-constrictors, but also an inhibition of vaso-dilators. This last fact is the most difficult to demonstrate, because it requires the presence of tone in the dilator centre, which is not the usual state of affairs. A similar difficulty was met with by Sherrington in certain of his experiments.

The effect of strychnine was found to consist in a conversion of the inhibitory component of both kinds of reflex into an excitation, while that of chloroform is of the opposite kind, namely, a conversion of excitation into inhibition. These effects of the two drugs were found to explain all the puzzling results met with after evisceration, and other interferences. In the eviscerated animal, with all the accessible vaso-constrictor nerves also cut, we have a preparation in which all the vascular reflexes are effected by the vaso-dilator centre. Since in the depressor reflex, there is excitation of this centre, it is easy to see that strychnine cannot reverse the depressor in these circumstances. Pressor reflexes, in which there is normally an inhibition of the dilator centre, become, after strychnine in the "dilator" preparation, depressor

in nature, owing to conversion to excitation of the dilator centre.

I venture to think that E. K. Martin in a recent paper has misunderstood the evidence for reciprocal innervation. It is not merely a question of balancing a pressor against a depressor response, but of the fact that in say a depressor reflex, produced in the same way in both cases, it can be shown that there is brought about both excitation of vaso-dilator nerves and inhibition of vaso-constrictor tone. The relative importance of the two in any particular organ depends on the preponderance of the one or the other kind of innervation.

Before leaving the subject of vascular reflexes, I would call attention to a not-infrequent source of error in the interpretation of peripheral effects. This is the failure to realize that a region which is larger than another or more copiously supplied with vaso-motor nerves, may affect this other by draining away or supplying more blood; so that, for example, if there is simultaneous reflex constriction in the viscera and in the skin, it may appear as if there were dilatation, or absence of effect in the skin, whereas repetition of the experiment in the eviscerated animal, or with an arrangement to prevent rise or fall of general blood pressure, shows that there was really constriction in both. Unless this test is applied, no conclusion as to opposite effects in the splanchnic and limb circulations can be made, although it is often done.

The peculiar nature of the vaso-dilator innervation of certain regions of the body may next attract our attention. Stricker in 1876 stated that when the peripheral ends of the posterior roots of the sacral plexus in the dog were stimulated, the temperature of the paws rose owing to vascular dilatation. Although this result was confirmed by some later workers, it was not generally accepted, probably because the methods adopted did not seem sufficiently free from objection to warrant acceptance of a contradiction of the Bell-Magendie law. At a still later date, when investigating the course of the vaso-dilator fibres of the hind leg by the plethysmographic method, I took occasion to test the effect of stimulating the dorsal roots and found to my surprise that Stricker's statement was quite correct, even when the spinal cord was cut out and mechanical stimuli used, so that all possible error seemed to be obviated. I was inclined at first to attribute the result to aberrant spinal efferent fibres, until I found that the effect was not abolished by section of the posterior roots between the ganglion and the cord and subsequent degeneration of any possible spinal efferent fibres. They did degenerate, however, when the ganglia were removed. Thus, the fibres in question were anatomically identical with the ordinary sensory fibres. At Prof. Langley's suggestion, the effect was called "antidromic," because of its being due to impulses passing along the sensory fibres in a direction opposite to the usually accepted one. The nature of the peripheral connections seemed puzzling and I suggested the possibility

of a peripheral plexus from which fibres passed on the one hand to arterioles, on the other to sense receptors, but thought it improbable on account of the reaction time being expected to be short on such a view. However, subsequently, Ninian Bruce showed that the process had the characteristics of an "axon-reflex" in the sense of Langley and Anderson, as it would be on the hypothesis mentioned, although a branched fibre would suffice, without the peripheral plexus. The facts brought out in Bruce's work are of interest in themselves. Spiess had shown that the irritant effect of oil of mustard on the skin is absent if cocaine has been applied. Since cocaine paralyzes sensory endings, it was thought that a spinal reflex of a vaso-dilator nature was involved. Bruce found on the contrary that the irritant effect was still present and paralyzed by cocaine if the trunk of the nerve to the region was cut. It was therefore independent of the spinal cord, not a reflex. It was abolished, however, if the nerve was allowed to degenerate. No other explanation than that of an axon-reflex is possible. Impulses pass from a sensory receptor up the nerve as far as the point where a branch is given off to an arteriole and then pass both onwards to the cord and backward to the blood vessels. Innervation from the cord passes both to the arterioles and to the sensory organ, but presumably does not reach beyond some synapse in the latter place.

This antidromic axon-reflex effect has recently become of interest in connection with the innervation of the capillaries. There was at one time felt to be a difficulty in supposing independent contractility of these vessels on account of their protoplasmic nature. But this is not serious, since cells like those of the amoeba are able to change their shape, and this is all that is needed. Roy and Graham Brown saw neighboring capillaries change their diameter independently of one another, so that the effect could not be one of mere passive response to arterial pressure. Krogh has recently investigated the matter in more detail. He found that stimulation by touching with a pointed needle caused a local dilatation, which spread along the capillary to a little distance. After the application of cocaine or degeneration of the nerve supply, the effect was limited to the point stimulated. The similarity to the antidromic phenomenon described previously is obvious and Krogh interprets it in that way. Starling has suggested that the whole of the antidromic effect might be situated in the capillaries. Under his direction, Doi made some experiments on the question. He found that the dorsal roots of the frog have an antidromic dilator effect; earlier observers had overlooked the fact on account of the very slow time course. Doi showed that it was still present after maximal dilatation of the arterioles by acetylcholine or of the capillaries by histamine. Hence it appears to be common to both.

The action of histamine, as investigated by Dale and Richards, has thrown much light on the properties of the capillaries. Dr. Dale has given an account of this work

in a previous Herter lecture, so that I may content myself with reference to its property of dilating capillaries, while causing contraction of plain muscle, including that of the arterioles. An important additional effect of histamine in large doses is to increase the permeability of the capillaries. This has its significance in respect of the symptoms present in severe wound-shock, as we shall see later.

Antidromic phenomena have a clinical interest in respect to Herpes. The work of Head and Campbell located the lesion in the dorsal root ganglia. The problem arises as to how far mere vascular dilatation can produce destruction of tissue, such as blisters and so on. Does persistent irritation of a nerve cause violent peripheral effects? This seems to suggest the need of experimental work.

Some recent work by Burn on pilocarpine has emphasized the importance of the capillary circulation for normal peripheral effects. The failure of the sweat glands to respond to this drug in certain nervous conditions has been a puzzle. Burn found that section and degeneration of the sympathetic supply to the cat's paw did not affect the response, whereas that of the main sciatic nerve usually abolished it. The reaction of the capillaries to histamine was also absent whenever that to pilocarpine was absent. In the course of regeneration, the reaction to pilocarpine returns at an early date, corresponding to that found by Head for the protopathic sensory fibres and much earlier than that of the sympathetic, motor, or epicritic fibres. In some experiments made by Head and myself, we found that the antidromic vascular dilatation from the peripheral end of the regenerating radial nerve was present at a time when only protopathic fibres had made their appearance. The facts as a whole suggest that the antidromic innervation of the peripheral circulation is necessary to the normal activity, not only of the capillaries themselves, but indirectly of the tissues in general.

I wish next to call attention to an aspect of the circulation which is apt to be unduly neglected, namely, the results which follow from changes in the volume of the blood in relation to the capacity of the vascular system which contains it.

Let us recall the fact that the amount of blood propelled by the heart depends on that reaching it by the veins, or in other words, on how much the ventricles contain at the moment of systole. It is clear that with a given total volume of blood, the more is contained in peripheral regions, the less must be in the heart. Hence, the amount in effective circulation is diminished and the arterial pressure is low. This aspect of capacity of the vascular system was always insisted upon by the Ludwig school and work done by Starling and myself confirmed their views. Mere consideration of rate of flow through dilated or constricted arterioles may lead to mistaken conclusions. For example, depressor stimulation causes an increase in this rate of flow and it might be expected to

cause a rise in the pressure in the vena cava, along with increased inflow into the heart and so on. This is not the case, however, except for a brief period; the increased capacity of the arterial system more than compensates for the increased rate of flow. Blood is held back in the enlarged arterial space.

This aspect of the circulation has assumed practical importance in relation to wound-shock, owing mainly to the work of Dale and Richards on the capillaries. When we remember the relatively enormous proportion of the capillary part of the vascular system to the whole, we realize how a comparatively small dilatation of these vessels, if widespread, may lead to a very serious drainage of blood into them, with loss of blood from effective circulation and all the consequences of deficient supply to vital organs. Practically all the symptoms of wound-shock can be accounted for on this basis.

Observations by means of the vital red method on men wounded in the war showed a deficiency of blood in circulation. This was due partly to capillary dilatation; partly, in later severe stages, to loss of plasma owing to the capillaries becoming permeable not only to salts but also to colloids. The general effect is similar to that of hæmorrhage and it was often impossible to distinguish between them.

So long as the increased permeability had not progressed too far, treatment of hæmorrhage and shock without hæmorrhage by transfusion of blood or by 6 per cent solution of gum acacia in 0.9 per cent sodium chloride was found effective.

The necessity of a colloid with an osmotic pressure equal to that of the blood proteins will be obvious from what was said in my first lecture. Salt solutions rapidly pass out from the blood vessels. The osmotic pressure of the colloids in blood or in gum-saline causes the fluid injected to be retained. [Tracings were shown of the different results of the two kinds of solution.] It is plain that unlimited loss of blood cannot be replaced by any solution devoid of hæmoglobin. The limit was found experimentally to be about when half of the blood had been removed. Such cases practically do not occur in practice and the experiments of Haggard and Henderson lately published do not bear upon the problem of the practical use of gum-saline, since in their experiments the hæmorrhage was in excess of that claimed to be capable of replacement. It is only in cases of excessive loss of blood that the factor of importance is the hæmoglobin. The real factor in other cases is the volume of the blood.

Experimental work on cats showed that gum-saline was effective in the state of shock produced by massive injury to muscular tissue. This was also found to be the case in many instances of wounds in the war. But there was a general impression that after the state of increased permeability had developed, treatment was hopeless. It was found that even blood left the circulation rapidly and was useless. But, at the same time, certain experiments

which I made on cats in histamine shock gave me the impression that if repeated injections of moderate volume at half-hour intervals or so had been tried, more favorable results would have been obtained. It appears that the first injections, although more or less rapidly lost, set the blood vessels by improved circulation through them on the way to recovery. Incidentally, gum-saline has an advantage here, since it is scarcely possible to arrange for repeated transfusions of blood.

Naturally, the hæmoglobin left in the circulation is diluted by an artificial fluid and it is perhaps surprising that the result is as good as it is. The explanation lies no doubt in the fact brought out by Gesell, namely, that the actual amount of hæmoglobin, etc., carried through a tissue in a given time is really increased. The greater rate of flow, owing to the larger volume in circulation more than compensates for the dilution. The "nutrient flow," as Gesell calls it, is increased.

After careful consideration of all the objections brought against the use of gum-saline, I am convinced that any unfavorable results are due either to non-observance of the methods which I have given for the making of the solution, or to its use in cases in which nothing whatever would have served. Of course, it cannot be expected to replace unlimited loss of blood. But, as much as two-thirds of that in the body can be effectively replaced. This implies a loss of so great a magnitude as rarely happens.

It seems probable that determinations of the blood volume will be found to give valuable clinical information. It is not necessary to use vital-red; the observations of D. T. Harris and others have shown that the easily accessible congo-red is really superior, since it leaves the blood vessels less rapidly. Owing to its cheapness, it can be purified, since the considerable incidental loss is immaterial.

The last matter to which I would refer, is some recent work on functional vascular dilatation. The increased supply of blood required by an active organ may be afforded in two ways—either by stimulation of vaso-dilator nerves from the centre, or by some chemical products formed by the activity of the cells. Since it is certain that the latter takes place, it has been thought by some that there is no need for the assumption of vaso-dilator nerves. I showed that carbonic acid, a universal product of cell activity, causes dilatation of the blood vessels. Gaskell had already shown that lactic acid acts in the same way, and lactic acid has recently been shown to be present in the venous blood from the tongue when its muscle contracts in response to stimulation of the hypoglossal nerve. The effect seems to be a general property of acids, that is of hydrogen-ion. From certain remarks made by Barcroft, a nitrogenous product of the nature of histamine appears to be in his mind. As to this, we have no evidence of a nitrogenous product in the normal activity of muscle. Moreover, capillary dilatation would be ineffective without arterial dilatation. In fact, acetyl-

choline would be appropriate. But we have as yet no evidence that it plays a part.

In this connection, two kinds of action of external cold on the skin are to the point. One of these, which may be regarded as the healthy reaction, is a red warm skin. There is arterial dilatation, which sends a rapid current of warm oxygenated blood through the capillaries and protects the skin from frost-bite. In the other, abnormal, reaction, the capillaries are also filled with blood, but the skin is blue and cold. Although the capillaries are dilated, the arterioles are contracted, so that the current of blood is slow, it becomes cold and loses its oxygen, becoming venous and blue. Capillary dilatation alone is thus insufficient to ensure a good circulation. I am inclined to think that the fall of blood pressure caused by histamine is due chiefly to a capacity effect of draining blood into the capillary region, and only to a small degree to a lowered peripheral resistance. A slight increase in the wide bed of the capillaries would have but little effect on the resistance, in comparison with that produced by changes in the arterioles.

There is also evidence that lactic acid is formed in the asphyxial condition of cells. If this compound is a stage in the oxidation of glucose, it is quite likely to remain as such in the absence of sufficient oxygen to burn it completely. The vaso-dilatation produced by this acid has its interest in relation to the response of arterial muscle to changes of internal pressure. My original experiments were made before the discovery of the reflex liberation of adrenaline by stimulation of sensory nerves, and were

inadequate to prove the reaction to stretching by rise of pressure. The dilator response to lowered pressure may have been due to asphyxial products, but in view of the conditions of the experiments, I think that this explanation is not altogether satisfactory. Further experiments on isolated arteries are needed, but my experience has been that it is a matter of difficulty to obtain these in a sufficiently responsive state. They readily pass into a contracted state, in which they seem inexcitable.

The actual existence of vaso-dilator nerves apart from "metabolites" has been demonstrated by some recent experiments by Anrep and Evans and by D. T. Harris. It was found that the lingual nerve produced a large dilatation in the tongue without any increase in oxygen consumption; that is, without any increase in cell activity. In fact, in Harris's experiments, brief stimulation of the lingual caused a much greater dilatation than a corresponding one of the hypoglossal, whose effect is only caused by products of activity of the muscle. The dilator nerves of the dog's tongue come into play especially in temperature regulation.

It is an interesting question whether innervation of dilator nerves is a part of the coordinated reflex to various effector organs, gland or muscle. Anrep found that the reflex dilatation of the submaxillary gland, brought about by application of acid to the tongue, was as great after atropine as before. Hence, the vaso-dilator mechanism was set in action reflexly, apart from the production of "metabolites."

NOTES ON NEW BOOKS

A Textbook of Gynecological Surgery. By BERKELEY and BONNEY. 829 pp. 2nd edition. Cloth, \$11.00 (N. Y., Paul B. Hoeber, 1920.)

It is a pleasure to turn over the pages of a book that is as well prepared as this one. The subject matter is for the most part presented in an up-to-date and clean-cut manner. The illustrations, although not elaborate, on the whole are clear and supplement the text well. To a large extent one of the commonest faults of recent gynecological text-books has been avoided—the tendency to distort the sense of balance and proportion by laying undue stress on minor pet procedures in which the authors are particularly interested. The sections on post-operative complications and care and on ultimate results add materially to the value of the work.

As the authors state, this book presents the opinions and practices of the school of gynecology to which they belong. In general, we feel that these views are sound and trustworthy, although quite naturally ours differ from theirs in some particulars. Thus, in our opinion they have underestimated the advantages that may at times be gained by draining the pelvis through the cul-de-sac of Douglas rather than through the abdomen, and they have also failed to appreciate the value of posterior colpotomy as a diagnostic or therapeutic procedure in selected cases. We would criticize their treatment of perineal plastic surgery as being incomplete. It seems unusual that more space should be devoted to the directions for dilating

the cervix (fourteen pages) than to the treatment of vesico-vaginal fistula (twelve pages). Little or no attention is paid to the subvesical fascia in the treatment of cystocele, vesico-vaginal fistula or prolapsus; this may be the cause of some of the failures that they seem to have had. The treatment of complete laceration of the perineum is too cursory to be sufficient; not enough attention is paid to the rectal sphincter or the obtaining of continence, which is usually the only indication for performing the operation. In the treatment of carcinoma of cervix, the authors are far more radical than are most gynecologists of the present day; it is an open question whether the ultra-radical operation involving the wide dissection of the pelvic and iliac glands, the parametrium and vagina is worth while. Their method of taking care of the vagina both before and after perineal operations is rather elaborate; in our experience perineal infections have been much less common since we have simplified our technique. We would question the wisdom of devoting space to operative procedures that are definitely out of date, such as Le Fort's operation for uterine prolapse or the making of a common cloaca out of the vagina and bladder in some cases of vesico-vaginal fistula. Their indications for performing hysterotomy and utriculoplasty also seem questionable. In their treatment of carcinoma of the rectum, they recommend that the combined abdominal and perineal operation be performed in one stage; in some cases, at any rate, it is undoubtedly safer to carry this out in two stages.

From what we have said, we would not wish the reader to get the idea that this is not a good book. On the contrary, it strikes us as being one of the best recent text-books on operative gynecology. With the above reservations, we can recommend it both to the expert gynecologist and to the beginner, for both will find in its pages much that is interesting and instructive.

L. R. W.

The Diseases of Children. By SIR JAMES F. GOODHART and GEORGE FREDERICK STILL. 11th Edition. Cloth, \$10. (New York, Paul B. Hoeber, 1921.)

This is the sort of book which is and was meant to be a text-book of pediatrics for students of medicine and should be judged as such. How well it has served its purpose in the past is indicated by the fact that this is the eleventh edition since it first made its appearance in 1885. In this last revision of the book Dr. Still has studied to retain as much of the text left by Sir James Goodhart as possible and still to bring it abreast of the present day.

The general consideration of the diet and hygiene of the healthy child is very well done and the author offers a reasonably simple, if somewhat dogmatic, system for the modification of milk for the hand-fed infant. The book is untouched by post-war fads which have grown up out of plausible empiricism masquerading as science. Their omission may stamp the book as obsolete to the beginner in whose novitiate they had their rise and before whose heyday they will pass into the limbo of the forgotten. After all, the question which matters in feeding a baby is not what *may* be given but what is the *safest* food for the average child. To teach this is all that can be attempted in a text-book. Experience only can teach a student the art of dealing with the idiosyncrasy of the individual child so as to secure optimal results. Still's feeding is based on the percentage composition of milk, and his formulæ are made up with added cream. An infant fed according to this book would receive a good bit more fat than is usually allowed by American pediatricists nowadays and the interval between the feedings is shorter than that which has found favor in this country. Nevertheless, the book offers the student a tolerable ground on which to base further study.

The pathology, symptomatology and diagnosis of the morbid conditions of childhood have been thoroughly and carefully set forth. Considerable less emphasis has been placed by the author on laboratory diagnosis than is found in most American books on medicine. As regards etiology, the book is not equally good throughout. For example, the author has gone into the etiology of poliomyelitis with some care and has reported it at sufficient length. On the other hand, if the text may be used as an index, he has entirely ignored the recent work which has been done to reveal the factors which underlie the occurrence of tetany. The chapters on the exanthemata are excellent.

In the treatment of the disease the author is much more prone to rely on administration of drugs than is customary in this country, possibly because he knows them better. There can be no doubt that in the hands of a competent therapist the simples and compounds of the pharmacist contribute much to the safe and happy curing of disease, and the list of prescriptions at the end of the book contains many excellent formulæ. Drugging is, however, easily overdone and many of the diseases

to which children are subject may be confidently and safely handled by regulating the diet and hygiene of the patients. It is well to remember that if one is to have recourse at all to the pharmacopeia in the practice of pediatrics, one should be qualified by a much greater knowledge of pharmacy, pharmacology and therapeutics than the average physician possesses.

*Diluis helleborum, certo compescere puncto
Nescias examen—*

In closing, a protest is in order against the exorbitant prices which are charged in America for English books. Ten dollars is far too high a price for this text which, although it is printed well and on good paper, is not expensively illustrated and is wretchedly bound with the cheapest possible material. In such a dress the book would be dear at half the price now charged for it. Not only should books be cheaper but they should be better made. By far the greater number of those published now will be dust before fifty years are past and the book which is worth publishing at all is worthy of publication with a view to a longer life. It may be that the useful existence of a modern book of medicine is but seven years. Granted that this is so, such a statement only supplies an argument for the preservation against time of the only available records of the period of change and investigation which is responsible for its truth.

P. G. S.

Modern Surgery, General and Operative. By JOHN CHALMERS DaCOSTA. Cloth, \$8.00. (Philadelphia and London, W. B. Saunders Co., 1919.)

The appearance of a new edition of DaCosta is always most welcome. Little comment is necessary or desirable on this wonderfully concise, accurate and up-to-date volume. For a subject so extensive, it is always a marvel how it can be done so well.

W. E. D.

Submucous Resection of the Nasal Septum. By WILLIAM MEDAUGH DUNNING, M.D. (New York, Surgery Publishing Company, 1922.)

This book contains forty-seven pages of text and twenty-five illustrations. The author discusses briefly the anatomy and physiology of the nose, and considers, in a somewhat cursory manner, the etiology of septal deviation and its relation to such general disorders as hay fever and asthma. He classifies septal deviations as (1) bowed or curved, and (2) angular, and describes the operative procedures he has devised for their correction. The schematic drawings illustrating the various steps of the operation could be improved upon. At the end he presents a few case histories which show the beneficial effects that result when the operation is really indicated and is properly performed.

The facts could have been presented in a much more concise manner, and while there are many interesting and helpful suggestions, there is really little that has been added to what we find in the standard text-books on rhinology.

J. W. B.

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THE DIAGNOSTIC AND THERAPEUTIC USE OF UVEAL PIGMENT IN INJURIES OF THE UVEAL TRACT AND SYMPATHETIC OPHTHALMIA

By ALAN C. WOODS, M. D. and ARNOLD KNAPP, M. D.

(From the Departments of Pathology and Ophthalmology of the
Johns Hopkins Hospital and University, and from the
Herman Knapp Memorial Hospital, New York)

In a former paper by one of us (Woods¹), clinical and experimental studies on the immune reactions following injuries to the uveal tract were presented. These studies showed that: (1) In an intra-ocular injury involving the uveal tract, where normal healing took place without the occurrence of a sympathetic disturbance in the fellow eye, substances developed in the blood serum which gave a positive complement fixation reaction with an antigen made from the pigment of the uveal tract. (2) In the cases of the intra-ocular injury involving the uveal tract

when normal healing was delayed, and where sympathetic disturbance in the second eye sometimes occurred, this complement fixation reaction to pigment antigen was absent. Furthermore, there was not only the absence of this complement fixation reaction, but in one case of sympathetic disturbance there appeared to be a definite hypersensitiveness to pigment. Evidence was also presented which had led the author to believe that the occurrence of this complement fixation reaction was evidence of the development of an immunity by the organ-

ism to the pigment, and gave definite protection against the outbreak of a sympathetic disturbance in the fellow eye. Likewise, it seemed clear that the failure to develop this complement fixation reaction was evidence of the failure to develop an immunity, and was so attended by a persistence of inflammatory symptoms and the liability to the development of a sympathetic disturbance in the fellow eye.

The possible clinical use of this phenomenon for the purpose of early information as to the prognosis, in any given case of intra-ocular injury involving the uveal tract, is at once evident. Should these facts hold true, the development in the blood serum of a positive complement fixation against pigment antigen would warrant the surgeon in giving a favorable prognosis, and allow him to leave the injured eye without fear of the development of a sympathetic disturbance in the fellow eye. On the other hand, the failure to develop a positive reaction would give definite information to the surgeon that sympathetic ophthalmia was an event to be feared, and would allow removal of the injured eye before the outbreak of the sympathetic disease.

Likewise, the possibility for the therapeutic use of uveal pigment in a sympathetic ophthalmia is manifest. As much as the hypersensitivity predisposes to the outbreak of the disease and an immunity protects against the disease, as soon as a hypersensitivity is demonstrable, the self-evident course is to desensitize the patient, and then, as a therapeutic measure, proceed either to active or passive immunization.

It is the purpose of this paper to report cases of intra-ocular trauma involving the uveal tract, in which this serum complement fixation reaction against uveal pigment was used as a diagnostic procedure to determine the status of the case with regards to a possible outbreak of sympathetic ophthalmia. Further, one case of malignant sympathetic ophthalmia is reported in which uveal pigment was used as a therapeutic procedure.

The Diagnostic Use of the Serum Complement Fixation Reaction against Uveal Pigment

The sera from seventeen cases of intra-ocular injury involving the uveal tract, occurring in the private and dispensary practices of the authors, were examined in the complement fixation reaction against an antigen of uveal pigment. The technique of the reactions and the preparation of the antigen have been previously reported.²

Grouping these cases according either to the results of the reaction or to the clinical picture and result, they fall into three rather general groups. The first group comprises ten cases which gave a positive complement fixation reaction, and in which healing took place without the occurrence of any sympathetic disturbance. In three of these cases, however, for one or another reason enucleation was performed. The second group comprises three

cases which all showed negative complement fixation reactions and at the same time showed more or less severe signs of a sympathetic disturbance. The third group comprises three cases which showed alarming symptoms in the injured eye, but without any manifestation of sympathetic disturbance in the second eye, and gave negative reactions.

GROUP I.—The following reports are of the cases in which the blood serum showed a positive complement fixation reaction against pigment antigen, following wounds of the uveal tract.

No. 1 (A).—Struck in the left eye April 16, 1921 by a piece of steel; the wound of entrance was at the lower corneal margin. The splinter of steel passed back through the iris and was localized by the X-ray, in the region of the lower ciliary processes. Size of splinter, 12x2 mm. Removed by magnet extraction April 17, 1921. Serum reaction against pigment antigen on May 2, 1921 was + + + and on May 21, 1921 + + -. The injured eye gradually underwent phthisis. In March 1922, the injured eye was small, sightless, but not inflamed. Right eye held 20/15 vision throughout and has remained free from trouble to date.

No. 2. (O).—Struck in right eye by a flying piece of steel on June 13, 1921. Wound of entrance at corneo-scleral border. The X-ray showed an intra-ocular splinter of steel, 13x3 mm. Successfully removed by magnet extraction on June 14, 1921. Serum reaction against pigment antigen on June 25, 1921, + + +. The injured eye healed quickly and retained 20/40 vision. The left eye held normal vision and has shown no subjective or objective signs of disturbance.

No. 3. (J.).—Struck in right eye on Sept. 14, 1921. Perforating wound of cornea with prolapse of iris and ciliary body. Prolapsed tissue excised within three hours after accident, scleral suture and conjunctival flap made. Uneventful healing, but a phthisical eye resulted. Serum reaction on Oct. 1, 1921, + + +. There was no disturbance of any kind in the left eye, but on Oct. 26, 1921, the right eye was enucleated for cosmetic reasons.

No. 4. (B).—Struck in right eye Dec. 14, 1921; the patient's spectacles were broken. There was a perforating wound of the right eye, involving cornea, iris, lens, and ciliary body. Prolapsed tissue excised after accident. Traumatic cataract developed and was removed.

The eye showed a continual cyclitis of varying intensity, which, finally, starting about the middle of February, gradually subsided with a retention of 20/50 vision, with correction.

The following were the serum reactions in this case:—Dec. 14, 1921, ±; Jan. 9, 1922, + +; Jan. 21, 1922, negative. Feb. 13, 1922, + + +; March 27, 1922, + + +.

Vision in the uninjured eye was 20/15 at the time of the accident. There has at no time been any failure of the visual acuity or field or disturbance of any kind.

This case is of especial interest in that recovery was delayed, and with this delay the patient passed from a positive phase to a negative phase and, finally, to a second positive phase which he has steadily maintained.

No. 5. (B).—November 1921, while hunting was struck in right eye with spent shot. Treated at home.

Exam. X-ray: Bullet down and in. Iridocyclitis and detached retina below.

Treatment: Enucleation. 12-11-21.

12-20-21. Discharged. Normal recovery.

12-11-21. Serum reaction against uveal pigment + +.

No. 6. (McC).—Nov. 15, 1921. Struck in left eye with a piece of glass.

Exam: x-shaped, penetrating wound in temp. side over region of ciliary body, the wound being 4-6 mm. long (Nov. 16, 1921).

Treatment: Scleral wound sutured—conjunctival flap.

Course: Looked very well for first ten days, then eye became red—ciliary congestion—iris showed greenish discoloration—Vit. exudate. Tension —.

12-9-21. Enucleation.

12-16-21. Discharged. Right eye normal.

Dec. 15, 1921. Serum reaction against uveal pigment, ±.

No. 7. (R.)—Dec. 21, 1921, while playing, was struck in eye by a knife.

Exam: Perforating wound of cornea starting a few millimetres above limbus at 12 o'clock and running downwards for about 8 mm. Iris cut above. Some vitreous in upper part of wound. Lens swollen.

Treatment: 12-28-21. Adm. to hospital. Wound cleaned up and some of cataractous lens removed.

Course: 1-6-22. Serum reaction against uveal pigment negative; no immunity.

2-4-22. Serum reaction against uveal pigment + + +.

2-17-22. Discharged. Ran through a long drawn out iridocyclitis with remissions and relapses. Finally left eye nearly white. Considerable cortex remaining. Capsule adherent to cornea and iris to capsule. V: R, 20/20; L, H. M.

3-20-22. Iridectomy.

4-15-22. Discharged. Normal recovery.

No. 8. (M.)—1-19-22. While on street was struck by some unknown object in right eye.

Exam: Linear incision through cornea from 9 to 5 o'clock and extending into ciliary body. Iris prolapsing, also part of ciliary body and some vitreous. V: L. P.

Treatment: Prolapsing iris, ciliary tissue, and vitreous, abscised. Wound closed with scleral and conjunctival flap.

Course: Normal recovery. Discharged 2-7-22. Vision 20/100.

When last seen in clinic V. 20/50 +. Eye was white. No pain, left eye normal. V: 20/30.

Serum reactions against uveal pigment:

2-1-22. Negative.

2-13-22. Anti-complementary.

2-28-22. +.

No. 9. (P.)—Sept. 1921, struck in right eye with splinter of steel. Three weeks ago noticed right pupil larger than left.

Exam: Corneal scar lower nasal quadrant with laceration of iris and opacity of lens directly behind. Embedded in retina, three disc diameters below disc there is an oblong-shaped foreign body surrounded by degenerated retina and choroid. Numerous small retinal exudates surrounding foreign body. V: 20/30.

Treatment: Giant magnet extraction through zonule and anterior cornea.

Course: 2-11-22. Serum reaction against uveal pigment, +.

2-17-22. Vitreous hazy. Some edema and cloudiness about old choroidal scar.

2-25-22. Discharged. V: 20/70. Eye white. Vit. clearer. Fundus unchanged.

3-16-22. Readmitted. Detachment of retina below. V: R, 10/200; L, 20/20.

Left eye normal.

No. 10. (P.S.)—January 2, 1922, left eye injured by wire in coasting. Prolapsed iris abscised. Eye has remained red. Shrunken. No pain.

Seen on February 6, 1922. Ciliary congestion. Iris discolored. Scar in cornea on temporal periphery. Somewhat indrawn. Beginning phthisis, faulty projection.

Serum reaction February 13, 1922, + + +.

No. 11. (B.)*—Feb. 2, 1922. Was struck in right eye by nail. He withdrew nail following accident.

Exam: V: H. M. Perforating wound of cornea in lower nasal quadrant. Iris cut and caught in wound. Lens cataractous. P. L., good.

Treatment: 2-6-22. Wound freed of iris.

Course: 2-14-22. Anterior synech. at site of injury, some exudate back of lens. Eye very red.

2-25-22. Serum reaction—negative. No immunity.

3-15-22. Discharged. Eye still very red. Tension normal. V: 10/200; L. proj., N; lens subluxated and cataractous.

4-18-22. Eye still red. No pain. Tension normal. V: H. M.; L. eye normal.

GROUP II.—The following reports are of cases in which following injuries of the uveal tract, the blood serum showed negative complement fixation reactions. These cases showed either at the time of serum reactions, or developing later, disturbances in the second eye which were believed to be sympathetic in nature.

No. 12. (H.)—Shot in head Aug. 30, 1921, with a shot-gun. Many shot penetrated skull and one penetrated left eye, passing through eye-ball and lodging near nasal wall of orbit. Craniotomy was performed Sept. 3, 1921 with removal of many shot. Iridectomy was performed on left eye on Sept. 9, 1921, for removal of iris tissue incarcerated in wound of entrance. Serum reaction on Sept. 8, 1921, negative. Serum reaction on Sept. 30, still negative. The eye was still inflamed, and sightless. Enucleation was advised and refused. The child returned to his home in Virginia on Oct. 1, 1921. On Oct. 7, an attack of photophobia with pericorneal flushing in the right eye was reported by the local physician. The child was brought back to Baltimore on Oct. 14, 1921. At that time there was still decided photophobia, but the eye was otherwise objectively negative. Serum reaction on Oct. 14, 1921, was negative. Enucleation of left eye was performed on Oct. 16, 1921. Following enucleation, the photophobia of the right eye disappeared, and the eye has remained entirely normal since then.

No. 13. (H.)—Penetrating wound of left eye in 1909. Normal healing with retention of 20/70 vision. In May, 1921, some solder flew up into left eye causing great pain which steadily persisted. Right vision began to fail in July, 1921. On examination, Aug. 23, 1921, the left eye showed a displaced pupil, back of which, bulging the iris forward, a foreign body could be distinctly seen. There was a low grade cyclitis; tension normal, vision 20/70. The right eye showed nothing objectively except marked vascular congestion of the fundus and slight photophobia. Vision was 20/30—with correction. Visual fields showed normal field in right eye and concentric contraction in left. A complete medical study at Johns Hopkins Hospital showed only negative results. Laryngological and all other special examinations were likewise negative. On Aug. 26, 1921, the serum reaction with pigment antigen was negative. A tentative diagnosis of sympathetic irritation in the right eye was made and enucleation of the left eye advised. The patient returned to his home in North Carolina where enucleation was performed, and he reported that following enucleation all symptoms in right eye cleared.

No. 14. (H.)—This case of malignant sympathetic ophthalmia was later treated by desensitization and immunization with uveal pigment. Full details are reported below. The first serum reaction on Dec. 21, 1921, at the height of the disease, was negative.

* Case incomplete—no serum reaction has been done since negative reaction of Feb. 25th. Case is still under observation, and is included in this series, inasmuch as the report includes all cases tested up to March 15, 1922.

GROUP III.—The following reports are of three cases in which the serum reaction was negative and in which the injured eyes were enucleated as a precautionary measure.

No. 15. (B).—Struck in left eye with a bit of steel Dec. 12, 1920; penetrating wound through ciliary body with intraocular retention of piece of steel. Removed by magnet extraction on the same day. Eye was sightless, and showed continual low grade cyclitis with periodic violent relapses. All medical examinations were negative. Radiograph showed no further intra-ocular foreign body. Jan. 17, 1921, and Feb. 15, 1921, the serum reaction against pigment antigen was negative. Left eye was enucleated on Feb. 21, 1921. Right eye normal.

No. 16. (K).—In Nov. 1911 was struck by a tree branch and suffered from perforating injury for which he was treated in his home town until Jan. 13, 1922.

Exam: Marked pericorneal congestion. Perforating corneal wound just below center. Iris greenish and adherent to wound. Pupil occluded. P: L., good. V: L., 20/20.

Treatment: Enucleation with fat implantation, 1-14-22.

Course: 1-26-22. Discharged. Normal recovery. V: L., 20/20. Serum reaction against uveal pigment, 1-20-22, negative.

No. 17. (P).—Struck in right eye with B.B. shot May 14, 1921. Shot penetrated globe through upper lid through sclera above cornea.

Treatment: Adm. to hospital and discharged as soon as hæmophthalmos had cleared up (6-9-21).

Course: Came to clinic for observation. Had several intra-ocular hæmorrhages. The tension gradually became soft and the eye remained red. Light projection faulty.

1-6-22. Serum reaction against uveal pigment, negative.

1-10-22. Adm. to hospital. Enucleation. Shot found outside globe, but adherent to it near optic nerve.

When last seen (4-18-22) left eye normal; V: 20/20.

There were two other cases in which all the serum reactions were negative, but these cases are of academic interest only. They were both instances of old sympathetic ophthalmia which had finally subsided following enucleation of the exciting eye. One case had followed sub-conjunctival scleral rupture and showed a sympathetic ophthalmia in the second eye which gradually recovered after enucleation of the injured eye. It is of interest that this patient showed a positive intradermal tuberculin reaction, confusing the diagnosis. The second case was one of delayed sympathetic ophthalmia in the left eye, following a bayonet wound of the right eye. When seen one year later, the left eye was nearly sightless. The right eye had been enucleated at the time of the sympathetic disturbance in the left eye.

COMMENT

In these cases above reported, it is frankly admitted that the presence or absence of a positive complement fixation reaction was not the sole index which governed the decision as to whether or not an injured eye should be left or enucleated. In two cases enucleation was performed before the blood was taken for the serum reaction, which in both cases was found positive. In other cases the presence of a positive serum reaction led to the temporary postponement of enucleation, which was later

permanently abandoned on account of the favorable outcome of the case. Nevertheless, an analysis of those cases which gave positive results, excluding the two cases in which the picture is confused owing to enucleation having formed prior to the discovery of a positive blood, shows a rather significant fact. Had we relied, in these cases, upon a positive serum reaction as the index of when not to remove an injured eye, our faith would have been justified. In other words, the occurrence of a positive serum reaction, irrespective of the condition of the injured eye at the time the reaction was done, was always followed in time by a subsidence of inflammation without any manifestation of sympathetic disturbance.

Whether the opposite of this is true—that a negative reaction is sufficient indication for removal of an injured eye for fear of a possible sympathetic ophthalmia—is not so clear. Of the seven patients who showed negative reactions, one is still under observation and three at the time of the serum test already showed, or quickly developed, signs of a sympathetic disturbance. In the remaining three cases, we did not wait for a possible sympathetic disturbance to develop—the injured eyes were enucleated. But these three cases all showed a long-standing, chronic cyclitis, the eyes clinically appearing dangerous. So in all three enucleation was justified by the clinical symptoms alone.

In the absolute interpretation of negative results, other factors must be considered. In the first place, the blood serum practically never becomes positive before ten days after the injury, and frequently never before four weeks. Furthermore, as is illustrated by case No. 4, Group I, the patient may go from a positive to a negative phase, and then finally to a positive phase. If a single or two negative reactions be taken as a definite indication for the removal of an injured eye, eyes may be needlessly removed, for in a few weeks more it is possible that the patient may go into a permanent positive phase, and be automatically protected against sympathetic ophthalmia. Therefore, if the serum reaction is negative, the time interval after injury must be first considered. However, if in six weeks after injury the patient has failed to develop a positive reaction, it seems unlikely, from the information we now have, that a positive reaction will ultimately develop. In such cases the possibility of sympathetic ophthalmia, and enucleation of the injured eye should be carefully considered.

There is one further point to be considered in the interpretation of positive results. Is the immunity indicated by a positive reaction permanent, or is the immunity likely to fail and the patient later become susceptible to sympathetic ophthalmia? This question is very difficult to answer. We have seen one, and only one, case go from an initial positive phase to a negative phase, and this patient later, within three weeks after entering the negative phase, again became positive, and has so remained.

An immunological fact is known which may be somewhat analogous to this reaction. In an animal experimentally immunized by repeated injections of foreign protein, each fresh injection is followed by a temporary decrease in the antibody titer. If the original titer is not very high, this decrease may throw the animal from a positive to a negative phase. This reaction is followed, however, by a further increase over the original titer, so that the animal soon again becomes more positive than before.

Every other case examined which showed a positive reaction, so far as our work has gone, has remained positive. Yet the following fact is true. In the earlier work on man with this complement fixation reaction, most of the patients tested had a traumatism that was one year or more old; and it must be admitted that positive reactions in these were weaker than in the more recent cases. From a review of the work previously reported, it seems clear that immunity, once definitely established, will last for from two to three years, and probably much longer, but gradually will become weaker.

Summarizing the results shown by the cases here reported which showed positive reactions, considered in the light of our previous studies, it seems probably that a positive reaction indicates a definite immunity to the development of sympathetic ophthalmia and that this immunity will last at least several years, if not permanently. From these findings it seems, then, that with the occurrence of a positive serum reaction, unless the clinical condition clearly contra-indicated such a course, the surgeon is warranted in not enucleating injured eyes.

The Therapeutic Use of Uveal Pigment

The basic points thus far emphasized in our work have been these: (1) The outbreak of sympathetic ophthalmia seems to be dependent upon an existing hyper-sensitivity to uveal pigment.¹ (2) The occurrence of an immunity against uveal pigment protects against the development of sympathetic ophthalmia. If these two fundamental points hold true, as it appears they do, they point at once to two very definite procedures—preventive and therapeutic—as regards sympathetic ophthalmia: (1) As a preventive or prophylaxis against sympathetic ophthalmia, the obvious procedure is to remove the hyper-sensitivity and produce the desired immunity. (2) As a therapeutic measure, to remove the one factor which appears essential to the outbreak of sympathetic ophthalmia, and to substitute for it the one factor that appears to make sympathetic ophthalmia impossible; in other words, again to remove the hyper-sensitivity and produce an immunity to uveal pigment.

With the use of uveal pigment as a prophylactic measure we have had no experience. The case reported below is also the only instance in which we have used the pigment as a therapeutic agent. It may be stated frankly that, inasmuch as this work is scarcely out of the experi-

mental stage, we felt considerable reluctance in using uveal pigment in man, knowing that a hypersensitivity to it existed, unless the circumstances surrounding the case were such as to make any procedure, offering the least hope, justifiable. The case below reported more than satisfied these conditions. Before any injections were made, Professor Hans Zinsser was good enough to consult with us, and it was only after this final consultation that the procedure was undertaken.

Report of a Case of Sympathetic Ophthalmia

The patient, a boy aged 8, seen on September 10, 1921, had an unhealed corneal ulcer, with prolapse of the iris in a dense scar, occupying the lower half of the right cornea, the result of a gonorrheal conjunctivitis, which had begun three months before. Vision was reduced to light perception and the tension was increased. The attempt to preserve the eye seemed justified; on September 13th, an iridectomy was done upward, the defect in the cornea was curetted, the prolapsed iris was removed as well as possible and the corneal wound covered with a conjunctival flap. This healed and the patient returned to his home after two weeks with vision of 2/200.

He returned on October 31st, because the other (left) eye had become red three days before. The left eye showed slight ciliary congestion, posterior corneal deposits, posterior synechiae. V: 20/20. Tension, normal. Interior, normal. The right eye was white. V: 2/200. The area of the corneal defect covered by a conjunctival flap was depressed and ocular tension was reduced.

There was no history of indigestion or overeating; the urine was normal; the stool showed evidence of intestinal fermentation, and the patient was placed on a sugar and starch-free diet and high colon irrigations. The eye was given the usual treatment. November 7th, V: 20/40; vitreous hazy; retinal veins engorged. November 9th, right eye enucleated. Serum reaction against uveal pigment was negative, showing no immunity whatever. A large cyanide subconjunctival injection was given; pilocarpine sweats and mercurial inunctions were begun. November 14th, atropin eczema. Left pupil contracting; nodules appeared in iris. Leucocytes, 3000; no lymphocytosis. December 8th, V: 20/40. Deep ant. chamber. Iris flat with nodules. Optic nerve blurred, retina hazy. Non-specific protein therapy was tried; a course of typhoid vaccines was given by Dr. Bonime. Though these were followed by the usual reactions, the leucocyte count did not increase and the eye continued to grow steadily worse. There was a marked follicular conjunctivitis with pseudomembranes. The general treatment was then changed to salicylate of soda in large doses. The eye showed more and more the characteristic changes of sympathetic ophthalmia with nodules in the iris; peripheric retraction of iris; complete synechiae and capsular opacity in the lens. On Jan. 5, 1922 the injections of uveal pigment were

commenced. During the period of treatment with the pigment, the progress of the eye disease did not seem to be particularly changed. Anterior chamber shallow; pupil contracting; iris uneven, flat total adhesion; tension increased. The last pigment injection was given Jan. 12.

January 18, 1922.—The eye was better; cornea and iris were clearer. T. 36. This improvement was only of brief duration and an exacerbation occurred on February 2, 1922, when the eye became more red, harder, and there was more exudation in the anterior chamber and in the iris. On Jan. 20, 1922, eight days after the course of immunization had been completed, the serum reaction had become ++—almost complete fixation. Following the exacerbation of Feb. 2, the eye gradually improved, though the opacity of the lens remained about stationary; iris retracted with some anterior peripheric adhesions; capsular opacities. On the last examination, the tension was normal and the eye white, V: 20/200. Details in fundus not visible. The blood examination on Feb. 8 gave + + +, showing complete fixation.

TECHNIQUE OF TREATMENT

Procedure with uveal pigment.—The suspension of pigment used in the case was made up as before described (2) and preserved with 0.15 per cent tri-cresol. As the pigment is de-naturalized by heat, unheated pigment was given, the only step taken for sterilization being the addition of tri-cresol, which is sufficient to destroy all ordinary non-spore forming pathogenic organisms. Since the critical condition of the patient did not allow sufficient time for animal experiments to exclude the remote possibility of the presence of tetanus spores in the preparation of pigment, it was decided to administer tetanus anti-toxin as a prophylactic measure. A precautionary intra-dermal test was made with tetanus anti-toxin to determine whether the patient was sensitive to the serum. This was positive, and he was first desensitized with serum in the usual manner, and then given 1500 units of tetanus anti-toxin. An intra-dermal skin test with uveal pigment was then made.

Technique of Intra-dermal test (Jan. 6, 1922).—Three different dilutions of the normal pigment suspension (the pigment of the uveal tract of an eye of a cow to 7.5 c.c. of salt solution is called "normal,") were used. The solutions were 1:100, 1:50, and 1:10. "normal," were used. The solutions were 1:100, 1:50, and 1:10. Through a fine hypodermic needle small intra-dermal injections, sufficient to raise a bleb about one half the diameter of a dime, were made with each dilution on the flexor surface of the forearm. A control was made with 0.15 per cent tri-cresol in normal salt solution. At the end of one hour the three blebs where the pigment suspension had been injected were surrounded by a striking, confluent urticarial wheal, whereas the control was practically negative. Within six hours this urticarial reaction had disappeared. Since the patient was strikingly hypersensitive to uveal pigment, we at once proceeded with desensitization.

Technique of desensitization (Jan. 6, 1922).—Intra-muscular injections in the buttock were made at two-hour intervals. The so-called "normal" suspension was used for the first four doses. The amounts given were 1.0 c.c.; 1.5 c.c.; 2.0 c.c. and 2.5 c.c. For the fifth injection a suspension of twice normal strength was used, 2.5 c.c. (the equivalent of 5.0 c.c. of the normal) were given. There was no essential change in either the blood pres-

sure, pulse, temperature or respiration during this period of desensitization. The only change observed was a generalized urticaria with the resultant unpleasant itching. This was relieved by a 70 per cent alcohol rub, and entirely passed within sixteen hours.

Technique of immunization.—On Jan. 6, during desensitization, a total of 12 c.c. of the normal suspension had been given, the last injection being the equivalent of 5.0 c.c. of the normal. On Jan. 9, the equivalent of 7.5 c.c. of the normal (condensed in a bulk of 2.0 c.c.) were given by intra-muscular injection into the buttock. On Jan. 12, after a second three-day interval, the final injection, the equivalent of 10 c.c. of the normal, was given by intra-muscular injection into the buttock. As for the previous injection, the pigment was condensed in a total volume of 2.0 c.c. No untoward symptoms accompanied either of the latter injections.

COMMENT

Sympathetic inflammation after perforating ulcers is rare. Peters comments on the fact that sympathetic ophthalmia has been frequently observed after perforation of a corneal ulcer complicating a gonorrheal process and concludes that the conditions in gonorrheal inflammation are more favorable to a development of sympathetic ophthalmia than in other keratitides. As an operation was performed in some of these cases, it may be that the prolapse is not so dangerous as the operation which was undertaken to remove it. The operations performed in these cases (quoted from Peters) were excision of the prolapse, (Schieck), cauterization (Trousseau, Gifford and Fuchs), abscission of corneal staphyloma and conjunctival suture (Fuchs).

In the case just described there seemed to be a definite intestinal autotoxemia. No change in diet or treatment, however, was of any avail. There was no lymphocytosis. Recent investigations have not shown the occurrence of a lymphocytosis in sympathetic ophthalmia. Treatment with typhoid vaccines was without avail. A leucocytosis could not be obtained.

Enucleation after the onset of the sympathetic ophthalmia was, as usual, without influence on the course of the irido-cyclitis in the remaining eye.

Microscopic examination of the eye first affected showed a characteristic diffuse infiltration of the iris and the adjoining iris angle, with mononuclear lymphocytes, a moderate infiltration of the ciliary processes and neighboring vitreous body, and only a few scattered foci in the choroid.

In view of the fact that partial recoveries have been observed in sympathetic ophthalmia, irrespective of the form of treatment, the inflammation having finally exhausted itself, it would be unwise to draw any deductions from a single experience. Yet in this case, after desensitization and immunization, the morbid process, with one slight and short period of exacerbation, came to a standstill; the eye became white and free from irritation and so remained.

SUMMARY

The immune reaction associated with intra-ocular injuries involving the uveal tract of the eye was made use of in seventeen such cases as a diagnostic procedure. In ten cases the complement fixation reaction was positive. These cases showed a normal healing without the occurrence of any sympathetic disturbance. One case is still incomplete. Three cases showed negative reactions, and of these one showed, clinically, a malignant sympathetic ophthalmia, and two showed definite signs of sympathetic irritation. The three remaining cases showed negative reactions and in these cases the injured eyes were enucleated as a precautionary measure. Two other cases of old sympathetic ophthalmia showed negative reactions.

The one case of malignant sympathetic ophthalmia showed, in addition to a negative blood picture, a positive skin reaction to the intradermal injection of pigment. In this case uveal pigment was used as a therapeutic agent. The patient was first desensitized to pigment and then actively immunized, with apparently beneficial results.

This case of sympathetic ophthalmia occurred, in a boy aged 8, after an operation for a perforated corneal ulcer with prolapse of iris after a gonorrheal ophthalmia. The inflammation pursued a steadily progressive course with all the symptoms of the severe type of sympathetic disease, despite all usual treatment—diet, intestinal irrigations, pilocarpin sweats, mercury inunctions, large doses of sodium salicylate, and non-specific protein therapy. Finally, following desensitization and immunization with uveal pigment, the process came to a stop, the eye became white, free from inflammation, the tension fell to normal, and has so remained. The process continued to be active for three months but has now been stationary for two months. Vision: 6/60. Peripheral retraction and flat total adhesion of iris; capsular opacities. Tension, normal.

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OBSERVATIONS ON THE PRODUCTION OF TOXIC SUBSTANCES BY PNEUMOCOCCI

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INTRODUCTION

In spite of the advances that have been made in our knowledge of the etiology and epidemiology of pneumococcus lobar pneumonia during the past decade, it must be admitted that but little progress has been effected in our understanding of the pathological physiology of the infection or of the factors that are responsible for the chain of events characterized by pneumococcus infection in man and to which we give the name lobar pneumonia. The common explanation for many of the events that take place during the course of lobar pneumonia is the assumption that along with the initiation of an inflammatory reaction in the lungs a substance or several substances are formed which are capable of being transported by the lymph or the circulating blood to distant portions of the body, there to bring about alterations in structure or function of vital organs. It is difficult to explain many of the phenomena of pneumonia without resort to this assumption, and it has therefore gained considerable favor with those who have observed the disease in man. That there is a profound alteration in the function of organs at some distance from the primary focus is not to be denied. Furthermore, the time-honored observations that the magnitude of these alterations is

often out of proportion to the extent of the pathological process, that they so often disappear abruptly at an appreciable interval of time before the local pathological process gives evidence on physical examination of beginning to clear up and while viable pneumococci may still be present in the lung tissue,¹ all support the conception that in pneumonia one has to deal with a toxæmia as well as a local infection and at times a bacteræmia.

The conception of the existence of a toxæmia in pneumonia is an attractive one, because it fits in so well with what one observes in the pneumonia patient, and is a relatively simple hypothesis. When, however, one attempts to test the validity of this hypothesis in the light of what is definitely known about pneumococcus infections, one realizes at once how difficult it is to bring forward in support of such a view evidence of an experimental nature which is at all convincing. It is impossible to say at the present time whether or not the pneumococcus through its own metabolic activity and independently of the host produces a toxin or toxins, or whether such a substance or substances are the joint product or products of a reaction between the parasite on the one hand and the cells of the host on the other. The latter conception has of recent years been gaining ground with some students of the infection.

HISTORICAL

Attempts to isolate toxic substances from pneumococci have been manifold. The earlier workers, following the lead given by the work on diphtheria toxin, sought for poisonous substances in the fluid portion of cultures of pneumococcus. The reported results are somewhat conflicting but in the main negative. Kruse,² in 1910, and Neufeld and Handel,³ in 1912, after a review of the earlier literature came to the conclusion that active poisons had not been obtained from the pneumococcus which could with any assurance be regarded as being identical with those which are believed to be present in pneumococcus infection in man and to give rise to the symptoms of general intoxication. Wadsworth⁴ concluded from his experiments that "dead pneumococcus culture material does not contain the active poisons formed in infection by living pneumococci."

Following Friedberger's work on the production of "bacterial anaphylatoxin" in the test-tube Neufeld and Dold⁵ produced a similar substance, using pneumococcus as antigen, and these same workers⁶ showed later that by extracting pneumococci in salt solution containing 0.1% lecithin toxic substances could be obtained, which, upon intravenous injection into guinea-pigs, resulted in death with symptoms of anaphylaxis. About the same time Rosenow,⁷ by extraction of pneumococci in salt solution for forty-eight hours at 37° C., secured solutions which on intravenous injection into guinea-pigs gave similar results. Cole⁸ worked with solutions obtained by dissolving pneumococci in sodium cholate. With such solutions injected intravenously into guinea-pigs he obtained anaphylactic-like symptoms including death with pathological findings similar to those encountered in serum anaphylaxis, and in the test-tube he was successful in producing lysis of red blood cells. Similar results were obtained by him with solutions of pneumococci obtained by freezing and grinding the bacteria. The hemolytic action of solutions of pneumococci could be inhibited by a serum prepared by injecting the solution into sheep and rabbits, as well as by minute amounts of cholesterin. Cole regarded the substances derived from pneumococci by procedures calculated to disrupt the cell as existing preformed in the bacterial cell and not as being the product of digestion as ordinarily understood by that term. He would class these substances as endotoxins.

S. Solis-Cohen, Weiss and Kolmer⁹ repeating Cole's experiments were "unable to produce uniformly a toxin which was hemolytic and also produced anaphylaxis on primary intravenous injection in guinea-pigs." Weiss,¹⁰ however, was able to produce it in sufficient quantities to arrive at the conclusion that it is very labile, that it is a true protein, that it inhibits the agglutinating action of antipneumococcal serum but is itself not affected by antipneumococcus serum, and that upon intracutaneous injection into sensitized guinea-pigs it produces an erythema and hemorrhagic edema in the subcutaneous

tissue overlying the muscle. From our own experience in working with sodium cholate solutions of pneumococci we can testify to the difficulty of securing a toxin from pneumococci by this method with any great degree of regularity. Because of the inconstancy of the results we were forced to abandon our attempts to study this substance.

The search for pneumococcus toxin has not been confined solely to the study of the organism itself. It is but natural that poisonous substances should be sought for in material derived from the pathological process as well. Issaef¹¹ found that filtered blood of infected animals as well as blood or peritoneal exudate sterilized by heating at 58° C. for two hours was toxic in amounts of one to two per cent of body-weight. The Klemperers¹² succeeded in killing young rabbits with the blood serum of infected rabbits in doses of two per cent of the body-weight. F. Klemperer¹³ found that the filtrate of the fresh pleural exudate of a dog was toxic for other dogs. Kruse and Pansini¹⁴ were not able to demonstrate toxic substances in the blood of infected animals, nor was Cole.¹⁵ The latter, however, found that the peritoneal exudates obtained from guinea-pigs dying after an intraperitoneal injection of pneumococci contained substances, which on intravenous injection into normal guinea-pigs gave rise to immediate symptoms like those seen in anaphylaxis and killed about one third of the animals with anaphylactic shock. Rosenow and Arkin,¹⁶ using extracts of pneumonic lungs and injecting normal dogs intravenously, produced changes which they regarded as similar to those seen in dogs dying after anaphylactic shock. Weiss, Kolmer and Steinfield¹⁷ investigated the toxicity of pneumonic lungs, and by subjecting the pathological tissue to pressure in a hydraulic press succeeded in obtaining a juice which, when sterilized by the addition of phenol or by heating to 56° C. for thirty minutes, was two to five times as toxic for mice, guinea-pigs and rabbits as was a juice obtained from normal human lungs treated in the same manner. Extracts of consolidated lungs in which the consolidation was produced by the intra-bronchial injection of aleuronat were in the main more toxic than extracts of normal lung and less toxic than those of pneumonic lungs. These extracts of pneumonic lungs were also found to be lytic for red blood cells. Manipulations, such as heating, drying and filtration through Berkefeld filters, decreased the toxicity of these extracts. It should be pointed out that the differences in toxicity of the extracts of pneumonic lungs and those of normal lungs were not so great as entirely to exclude the experimental error resident in all biological experimentation.

DISCUSSION

One may summarize the previous work on this phase of pneumococcus infection as follows: There is general agreement amongst the workers in this field that pneumococci or pathological material the result of pneumo-

coccus infection can be so manipulated as to yield (somewhat irregularly) substances which upon intravenous injection into laboratory animals give rise to symptoms and lesions which are identical with those observed after a second dose of protein-containing material has been administered to an animal previously sensitized to that protein. The correct interpretation of these facts is not so easy; their relation to pneumococcus infection in man is by no means clear, as Cole and others have pointed out. There is no direct evidence to show that the toxic substances obtained by manipulating the pneumococcal cells or exudate produced by them are ever formed in the human body during the course of pneumococcus infection. If it is true that they are formed solely as a result of solution of the bacterial cell, and if they are produced during the course of pneumonia and are responsible for the general toxic symptoms encountered in that disease, we must suppose that many pneumococci are destroyed in the lung during the course of pneumonia and even at the outset of the disease, for it is well known that the toxic symptoms of pneumonia are often quite severe at that time as well as later. To what extent extracellular destruction of pneumococci takes place in the consolidated lung is likely to remain a matter of conjecture and not susceptible of determination. Whether or not such a process does occur, it is certain that many of the organisms are taken up by the leucocytes and destroyed within the cells, and it is difficult to see how this intracellular process of destruction could result in the setting free of pneumococcus endotoxin unless the leucocytes can excrete such a substance without alteration. The work on agar anaphylatoxin and particularly the studies of Novy and his associates¹⁸ seem to us to do away with the idea that the animal effects observed after injection of pneumococcus toxin are specific or characteristic, no matter whether the toxin is obtained from the pneumococci themselves or from pathological exudates. In view of their work the query may be raised whether the occurrence of anaphylactic symptoms in guinea-pigs after intravenous injection is a sufficiently safe criterion for the determination of the toxicity of bacterial preparations. Further evidence of toxicity, such as might be elicited by subcutaneous or intraperitoneal injection, would seem to us desirable before concluding that one was dealing with a toxin capable of being formed in man in amounts sufficient to give symptoms. It should be noted here that Weiss, Kolmer and Steinfeld¹⁹ obtained deaths after intraperitoneal injection of the exudate from pneumonic lungs in amounts considerably less than those needed if cultures were used in the preparation of the toxin. It will be generally admitted that the toxic substances thus far described are of a low potency for the customary laboratory animals. This fact is evidence for the view that such substances play a slight, if any, rôle in the pneumococcus infection in man, although it is possible that a toxin of a low potency operating over a

prolonged interval of time may be just as deleterious as one of high potency acting over a short period.

It has seemed to us much more reasonable to associate the phenomena of intoxication in pneumonia with the growth of pneumococci rather than with their death and dissolution. When one examines the peritoneal exudate of mice dying after an intraperitoneal injection of living pneumococci, one sees evidence of tremendous proliferation of the bacteria and, as judged by the Gram stain, almost all appear to be in a healthy condition. Such mice invariably give a positive heart's blood culture. This same picture, moreover, is seen when mice are injected with sputum containing many other bacteria beside pneumococci. In such instances the pneumococci rapidly outstrip the other bacteria in their growth (frequently the latter are in great part destroyed) and one sees an overwhelming infection with pneumococci as if conditions were ideal for the growth and multiplication of those bacteria and not of the others. One certainly does not get the impression that pneumococci have been destroyed in great numbers in the peritoneal cavity of the mouse, and yet the mouse shows symptoms of intoxication and ultimately succumbs. It is difficult to conceive of rapid growth and rapid extra-cellular death of pneumococci occurring at the same time and in the same place, where conditions are as favorable for growth as they appear to be in the peritoneal cavity of mice.

If the toxic phenomena of pneumonia were associated with the growth and multiplication of pneumococci, it seemed to us conceivable that toxic substances might be present in a culture of pneumococcus while the organisms were growing rapidly, and that the chemical nature of such substances might be altered during the later stages of growth of the culture so that they would no longer be recognizable as such. In some studies on the nature of bacterial lag one of us²⁰ had been impressed by the fact that, when pneumococci are grown in meat infusion broth at 37° C., the maximum number of bacteria is attained within eighteen hours as a rule, sometimes earlier, and that after this period there is often a falling off in the number of living bacteria. The period during which pneumococci multiply at the maximum rate of growth in favorable fluid media is relatively short, not longer than twelve hours as a rule, and during this time significant changes take place in the H-ion concentration of the medium.²¹ It seemed to us that this might constitute a desirable time at which to search for pneumococcus toxin, since the more acid condition of the medium later on might bring about destruction of the relatively unstable substance formed during an earlier phase. Support for this view is offered by the work of De Kruif and Ireland,²² who have shown that the hemolysin produced by the streptococcus hemolyticus reaches its maximum at a very early period in the life of the culture, begins to disappear when the period of active growth ceases, and may disappear completely in fourteen hours. Zinsser and his

associates²³ have recently reported finding substances toxic for rabbits in cultures of a number of different species of bacteria and have found that the potency of these substances, while admittedly low, was in many instances greater in the case of young cultures, and appeared to diminish with age. They failed to find indication of the formation of such substances in the case of pneumococcus, type I, or staphylococcus aureus "in a few isolated experiments."

Because of the importance of the problem and because we have been interested in the question for several years, it seems to us desirable to extend these observations on the pneumococcus, making use not only of the ordinary fluid media but also of substances such as human serum and human blood, in order to approach as closely as possible conditions in the body.

Most workers in bacteriology have in the past dealt with cultures as old as or older than eighteen hours. They have lost sight of the fact that in fluid cultures of actively growing organisms the maximum number of bacteria is usually attained by that time, and that one is no longer dealing with a culture the members of which are multiplying actively. Chemical conditions in a culture of that age are quite different from those obtaining a very short while afterward.²⁴ To test the assumption that there might be found in fluid cultures of pneumococci, during the period of active growth, substances of a toxic nature, a series of experiments were carried out. It may be said in advance that they yielded only negative results. For that reason the results will not be given in detail, but only a general statement of the method and findings will be submitted for purposes of record.

TECHNIQUE

Two strains of pneumococci type I, isolated from recent cases of lobar pneumonia, were used. The media employed were: (1) beef infusion broth prepared according to the method described by Avery and Cullen;²⁵ (2) normal human serum; and (3) defibrinated human blood. The last two media were employed with the idea that conditions existing in the human body would be more closely approximated than in the case of beef infusion broth. Incubation was at 37° C. Specimens were removed at frequent intervals for filtration and plating for counts. Filtration was carried out with previously tested Berkefeld N candles. The clear filtrates were injected intraperitoneally into white mice in amounts of one cubic centimeter each. Bacterial counts served to show at what phase in the period of growth the specimens were removed.

RESULTS

In no instance were any toxic effects observed in the mice, no matter whether the specimens were obtained from the culture at the beginning, during the middle

or toward the end of the period of maximal rate of growth. Our experiments, therefore, were entirely negative so far as demonstrating the presence of a soluble toxin in the fluid portion of a rapidly growing culture of pneumococcus in beef infusion broth, normal human serum or defibrinated human blood. They cannot be said to throw any light on the complex problem of the cause of the intoxication in lobar pneumonia.

SUMMARY AND CONCLUSIONS

1. The relationship of the toxic substances obtained from pneumococci by solution of the bacterial cell or by manipulation of pneumonic exudates to the phenomena of intoxication in pneumonia in man is discussed.

2. Filtrates of actively growing cultures of pneumococci in beef infusion broth, normal human serum and defibrinated normal human blood are not toxic for mice when injected intraperitoneally.

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INFLUENZA-LIKE BACILLI

GROWTH OF INFLUENZA-LIKE BACILLI ON MEDIA CONTAINING ONLY AN AUTOCLAVE-LABILE SUBSTANCE AS AN ACCESSORY FOOD FACTOR

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For a long time, in spite of numerous studies, very little was definitely known about influenza bacilli. Practically every statement in regard to them has been challenged, leading to numerous controversies, one of which was whether they actually required hemoglobin for their growth. Pfeiffer¹ at one time thought that on a purely morphological basis he could distinguish pseudo-influenza bacilli from the true forms. Wolff,² in 1903, stated that Pfeiffer had decided his original idea of pseudo-influenza bacilli to be incorrect. Later, pertussis bacilli led to more confusion and some workers³ speak of pertussis-like bacilli without describing them accurately enough for others to recognize these organisms when found. When the pandemic of influenza came in 1918, *B. influenza* was characterized as a Gram-negative, non-motile, pleomorphic, aerobic, hemoglobinophilic bacillus. All strains were considered more or less alike serologically, regardless of morphology or virulence.

Since 1918 much profitable work has been done on influenza bacilli. It is known that there are hemolytic and non-hemolytic varieties, that they usually reduce nitrates to nitrites, that some form indole while others do not, that some ferment no sugars while others attack various carbohydrates. The most interesting work, however, has been that dealing with the growth requirements⁴⁻¹⁰ of these organisms. It has been definitely shown that true influenza bacilli require two accessory food substances, one of which resists autoclaving at 15 pounds for 30 minutes, while the other does not. Both substances are present in blood. They have been found elsewhere; the autoclave-stable factor in unfertilized eggs and potatoes, the autoclave-labile one in yeast extract, potatoes, tomatoes, beans and carrots. Respiratory strains differ culturally¹¹⁻¹³ and show a marked serologic heterogeneity.^{14, 15} This difference is not so evident in the group producing meningitis.¹⁶ Most of the meningitic strains examined were closely related culturally and serologically. *B. hemoglobinophilus canis*, described by Friedberger¹⁷ and placed in the hemophilic group, has been shown¹⁸ to require the addition of only the autoclave-stable substance as an accessory food factor. This bacillus has much in common with influenza bacilli, and several years ago it would not have been easy to differentiate it from them.

Has all this work made it possible to say that any given bacillus does or does not belong to the influenza group? Is there some one thing common to all of them

that no other group possesses? Are there organisms found in throat and lung cultures that are not influenza bacilli, yet which might be mistaken for them? Many of these questions can be answered now. Pertussis bacilli when first isolated are rather difficult to grow. Special media have to be used, but after the strains have been isolated for some time neither of the food accessory factors are required. *B. hemoglobinophilus canis* requires the addition of only the autoclave-stable substance. Hemolytic and non-hemolytic influenza bacilli, after from 2 to 7 years' cultivation on artificial media, still need both accessory food substances. Certain bacilli to be discussed in this paper, after 2 years' cultivation outside the body, will grow on media to which only the autoclave-labile factor has been added as an accessory food substance. These bacilli might be mistaken easily for influenza or pertussis bacilli and probably have been so mistaken in the past.

DESCRIPTION OF TWO INFLUENZA-LIKE BACILLI

Bacillus No. 1 was isolated from a throat culture of a patient with influenza in February, 1920. Bacillus No. 2 was isolated from the right lung of a patient who died of pneumonia following influenza in February, 1920. The throat culture of this patient yielded hemolytic streptococci and hemolytic influenza bacilli. A culture from the trachea showed hemolytic streptococci, hemolytic influenza and non-hemolytic influenza bacilli. From the right lung hemolytic streptococci were recovered in addition to bacillus No. 2. The non-hemolytic influenza bacillus recovered from the trachea was a Gram-negative, non-motile, pleomorphic bacillus which formed indole, reduced nitrates to nitrites, fermented no sugars, produced little or no change in blood-milk, and required both accessory food factors for growth.

Growth on Blood-Agar.—These influenza-like bacilli grow somewhat more slowly on blood-agar than ordinary influenza bacilli, but the colonies are enough like those of the latter to be mistaken for them. This is especially true of No. 2. Young colonies of No. 1 are regular, round, glassy and transparent. Older colonies have a brownish granular center and occasionally very old colonies have a crinkled surface with radiating folds (Fig. 1, 1a and 1b). The colonies of bacillus No. 2 are flatter, not so glassy, and have a more granular brownish center (Fig. 1, 2). The colonies of No. 1 are usually smaller and not so moist as those of No. 2. The difference in size of

colonies is not always so marked as in the figures. Colonies vary from 1 to 3 mm. in diameter. Both bacilli grow well under aerobic conditions.

Morphology, Staining, Motility.—The organisms are Gram-negative, non-motile, pleomorphic, non-sporebearing bacilli which cannot be differentiated from influenza bacilli morphologically. As a rule No. 1 (Fig. II) is smaller and more regular than No. 2 (Fig. III).

Hemolysis.—Neither strain hemolyzes red blood cells.

Production of Indole.—Neither bacillus forms indole.

Reduction of Nitrates.—Both bacilli reduce nitrates to nitrites.

Production of Amylase.—A starch-splitting ferment is not produced by either strain.

Action on Milk.—No marked change was noticed in the blood-milk tubes after incubation for 3 weeks.

Fermentation of Sugars.—The fluid and solid media for fermentation tests were prepared as described in a previous paper.¹⁶ Dextrose, sucrose, maltose and xylose were not fermented in a liquid medium by either bacillus. On a solid medium No. 1 showed a questionable acid formation from dextrose, sucrose and maltose.

Growth Requirements.—The autoclave-stable and the autoclave-labile growth accessory substances were prepared and used as indicated in a previous paper.¹⁸ The stable factor was hematin, the labile one was yeast extract sterilized by means of a Mandler filter.

The two influenza-like bacilli would not grow for more than a generation or two on meat extract agar, meat infusion agar, 2 per cent peptone agar, 2 per cent peptone agar and hematin, 2 per cent peptone water, 2 per cent peptone water and hematin, or 2 per cent peptone water and ascitic fluid. The results of these experiments are summarized in Table I.

TABLE I.
Growth Accessory Requirements of Certain So-called Hemophilic Bacilli.

Bacterium	Meat Extract Agar	Meat Infusion Agar	2% Peptone Agar	2% Peptone Agar and Hematin	2% Peptone Agar and Yeast Extract	2% Peptone Water and Yeast Extract	2% Peptone Water and Autoclaved Yeast Extract	2% Peptone Agar and Hematin and Yeast Extract
<i>B. pertussis</i> No. 256	+	+	+	+	+	+	+	+
<i>B. hemoglobinophilus canis</i>	—	—	—	+	—	—	—	+
<i>B. influenzae</i> , non-hemolytic	—	—	—	—	—	—	—	+
<i>B. influenzae</i> , hemolytic	—	—	—	—	—	—	—	+
Influenza-like bacilli Nos. 1 and 2	—	—	—	—	+	+	—	+

+ Indicates that successful transplants were made as long as desired. — Indicates that successful transplants were not obtained, or for one or two generations only.

In meat infusion broth these influenza-like bacilli grow well for one generation; after that none of the tubes showed any gross turbidity. If, however, amounts of 1 c.c. were inoculated daily from tube to tube, transplants

from the tubes to blood agar showed from 10 to 30 colonies regularly for many generations. Evidently there was a slight multiplication of the bacilli, or at least they did not die for a long time. This could not have been told from the gross appearance of the broth tubes and was discovered by making transplants to blood agar. The meat infusion broth was autoclaved. This discrepancy may be more apparent than real, for it may be possible for the heat-labile factor to withstand a short autoclaving without being entirely destroyed.

In Table II are listed the most important differential biological reactions of the hemophilic bacilli so far studied.

TABLE II.
Differential Biological Reactions of Certain So-called Hemophilic Bacilli

Bacterium	Reduction of Nitrates	Indole Production	Motility	Hemolysis of Red Blood Cells	Fermentation of Sugars	Action on Milk	Accessory Food Factors	
							Autoclave-Stable	Autoclave-Labile
<i>B. pertussis</i> not recently isolated	—	—	—	—	—	Strongly Alk.	—	—
<i>B. hemoglobinophilus canis</i>	+	+	—	—	+	Indefinite	+	—
Influenza-like bacilli Nos. 1 and 2	+	—	—	—	±	Indefinite	—	+
<i>B. influenzae</i> hemolytic	+	±	—	+	±	Indefinite	+	+
<i>B. influenzae</i> non-hemolytic	+	±	—	—	±	Indefinite	+	+

(Under nitrates) + indicates reduction, — indicates no reduction. (Under indole) — indicates no indole formation, + indicates indole formation, ± indicates indole may or may not be formed. (Under motility) — indicates lack of motility. (Under hemolysis) + indicates hemolysis takes place, — that hemolysis does not occur. (Under sugars) — indicates no sugars are fermented, + indicates that certain sugars are fermented, ± indicates that certain sugars may or may not be fermented. (Under food accessory factors) + indicates that the substance is essential, — indicates that the substance is not essential.

DISCUSSION

In another paper¹⁸ various ideas about the nature and the action of the two accessory food substances were discussed. At that time the question was raised as to whether there is an actual interaction between the two substances, as suggested by some observers, in promoting the growth of influenza bacilli. The question was raised then because *B. hemoglobinophilus canis* was found to require the addition of only the autoclave-stable factor. Evidence has been presented in this paper that certain influenza-like bacilli need the addition of only an autoclave-labile substance as an accessory growth factor. Yeast extract contains many other substances besides this heat-labile factor. It is not these substances, however, that are so essential because autoclaved yeast extract does not meet the needs of these bacilli whereas filter-sterilized extract does. It is fully realized here again

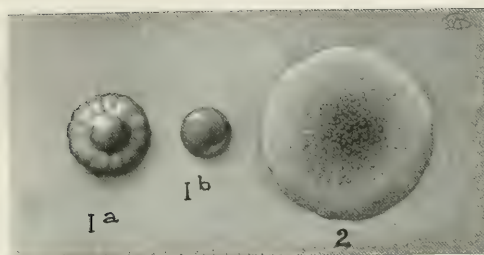


FIG. I

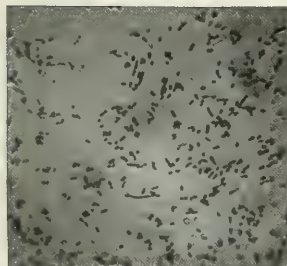


FIG. II

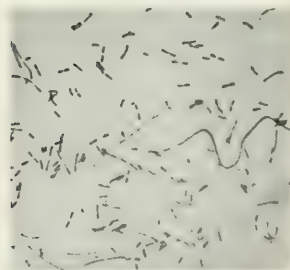


FIG. III

that one cannot argue conclusively by analogy from one group of organisms to another. Yet the fact that these influenza-like bacilli, as well as *B. hemoglobinophilus canis*, require only one of the accessory factors, casts further doubt upon the possibility of any interaction between the two accessory substances in promoting growth of true influenza bacilli.

Only two influenza-like bacilli have been described here; there are probably many more. From throat cultures taken during the epidemic of influenza in 1920 a number of atypical strains were obtained. Most of these were lost, as it is more difficult to cultivate the atypical strains than the typical ones on artificial media.

Stillman¹⁹ and others have recovered more than one kind of influenza bacillus from the same patient. The influenza-like bacillus, No. 2, was obtained from the lung of a patient who had true non-hemolytic and hemolytic influenza bacilli in the trachea and hemolytic influenza bacilli in the throat culture. No. 2 was considered an influenza bacillus for a long time as it would not grow on meat infusion agar. It was only after working out these strains carefully, particularly in regard to their growth requirements, that the difference was discovered. Until a few years ago the criteria by which an organism was judged to be an influenza bacillus were: Gram-negative, non-motile, pleomorphic, aerobic, hemoglobino-philic bacillus. The hemoglobino-philic qualities were tested on ordinary nutrient agar. Various workers must have had some mental conception of a true influenza bacillus because they were always speaking of typical and atypical ones. Most of this differentiation was made upon morphology alone. It is evident that many mistakes were made.

Is it possible to identify a given bacillus as a true *B. influenza*? Although it may be impossible to give a final answer to this question, one is better qualified to discuss it now than three or four years ago. It seems advisable to determine the accessory food factor requirements of each suspected bacillus, and to call only those true influenza bacilli which require both factors. The ones which require the addition of only the heat-stable factor have been named already by Friedberger.¹⁷ What, however, should be the designation of the influenza-like bacilli which need only the heat-labile accessory food factor as found in unautoclaved yeast extract? *Bacillus para-influenzae* or *Hemophilus para-influenzae* might serve as a good name for this group of organisms which are probably no more alike culturally or serologically than are the true influenza bacilli.

CONCLUSIONS

Two influenza-like bacilli have been described which require the addition of only an autoclave-labile substance as an accessory food factor.

Bacillus para-influenzae or *Hemophilus para-influenzae* has been suggested as the name for this group of organisms.

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EXPLANATION OF FIGURES

Fig. I.—1a, type of colony that occasionally appears in very old cultures of bacillus No. 1. 1b, usual type of colony in 48-hr. culture of bacillus No. 1. 2, usual type of colony in 48-hr. cultures of bacillus No. 2.

Fig. II.—Film from a 24-hr. culture of bacillus No. 1 on blood agar. (×1200.)

Fig. III.—Film from a 24-hr. culture of bacillus No. 2 on blood agar. (×1200.)

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A NEW METHOD OF TESTING LIVER FUNCTION WITH PHENOLTETRACHLORPHTHALEIN

SECOND COMMUNICATION

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Johns Hopkins University)

In a previous paper¹ the writer described a method of testing liver function by injecting phenoltetrachlorphthalein intravenously and studying its rate of disappearance from the blood stream. This was carried out in normal dogs, and in those with damaged livers. Under normal conditions, if the dosage is not too large, the liver is practically the only organ that excretes the dye, as was first shown by Abel and Rowntree.² Later Rowntree, Bloomfield and Hurwitz,³ and Whipple, Mason and Peigh-tal⁴ used phenoltetrachlorphthalein as a test for liver function by determining the amount excreted in the stools during the 48 hours subsequent to its injection intravenously. Further studies with this method were carried out by McLester and Frazier,⁵ Kahn and Johnston,⁶ Sisson,⁷ Chesney, Marshall and Rowntree⁸ and Krumbhaar.⁹ Their reports show results that are conflicting, and this method has not met with extensive clinical usage. Certain of its disadvantages were stated previously.¹ In 1916 McNeil¹⁰ introduced the method of testing liver function by determining the time required for the dye to appear in the bile, obtained through the duodenal tube. This procedure was given a recent trial by Aaron, Beck and Schneider¹¹ who used the Meltzer-Lyons method of bile drainage. Results by this method must be interpreted in terms of negative values; the test is not quantitative, and from experiments presented in this paper it is felt that the appearance time of tetrachlorphthalein in the bile is governed to a large extent by the condition of the bile-ducts, and does not necessarily depend upon the amount of functioning liver tissue present. By the author's method, results are quantitative, and, as is shown in the following experiments, they are within certain limitations independent of the condition of the bile channels; it is believed that the rate at which the liver removes this dye from the blood stream gives an index of the functional capacity of the entire organ.

When, owing to impaired function, the liver is unable normally to excrete this phthalein, it escapes in the urine, but does so with difficulty and quite slowly. This means of escape is apparently dependent upon the length of time that the dye remains in the blood stream, and upon the concentration that it reaches there. In the former experiments chloroform poisoning was used to produce liver injury;^{12, 13, 14, 15} when the impairment of function was greatest, as evidenced by the degree of jaundice and the toxic condition of the dogs, remarkably high curves were obtained. During the stage of repair, the concen-

tration that the dye reached in the plasma, and the time required for it to disappear from the blood stream, gradually decreased, until curves approaching normal were obtained after sufficient time had elapsed for restoration of function.

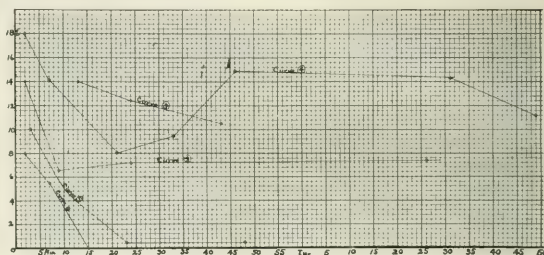


FIG. 1

Fig. 1 illustrates the very striking retention of the dye in the blood during the degenerative stage of chloroform poisoning. On the eleventh day of this experiment, although all objective signs and symptoms of impaired liver function had disappeared, the sensitiveness of the test is shown by the fact that the curve then obtained had not quite returned to normal.

SIMPLIFIED METHOD FOR QUANTITATIVE ESTIMATION

In these experiments the dose of tetrachlorphthalein employed was 5 mgs. per kilo. A sample specimen of blood was withdrawn before the injection; 2 c.c. of the plasma obtained were diluted with 5 c.c. of water, to which had been added a known quantity of the dye. This was compared colorimetrically with samples taken at frequent intervals following the injection. For convenience, results have been expressed in per cent of concentration, 10 mgs. of the dye to 100 c.c. of plasma being taken as 100-per-cent concentration. The technique was fully described in the former paper.

By this method, colorimetric readings below 10 per cent were often unsatisfactory, and hemolysis usually rendered comparison impossible. It was found that a series of standards could be made by adding concentrated solutions of the dye to the undiluted plasma, small test-tubes of uniform size being used. Eight cubic centimeters of blood are withdrawn before injection of the dye; standards are made from the plasma as follows:

TABLE I.

0.2 c.c. 100% dye sol. 0.6 c.c. plasma	0.2 c.c. 80% dye sol. 0.6 c.c. plasma	0.2 c.c. 60% dye sol. 0.6 c.c. plasma	0.2 c.c. 40% dye sol. 0.6 c.c. plasma	0.2 c.c. 20% dye sol. 0.6 c.c. plasma	0.2 c.c. 12% dye sol. 0.6 c.c. plasma
25%	20%	15%	10%	5%	3%

FIG. 2

The unknown plasma is alkalized with 3 drops of 5-per-cent sodium hydroxide, and without dilution is matched directly with the standards, using naked eye comparison in a good light. Two or three cubic centimeters of blood yield sufficient plasma for comparison. High readings by this method check with the colorimetric method closely enough for practical purposes; low ones are usually more accurate, and also the presence of hemoglobin proves less confusing. However, unless the plasma is free from bile and hemoglobin, readings below 5 per cent have been recorded as large, medium, or faint trace, and charted between 0.5 and 3.0 per cent.

A simple qualitative test, that shows up very faint traces of the dye, has also been worked out. To unalkalinized plasma several drops of 3-per-cent hydrochloric acid are added; this is carefully layered over a 5-per-cent solution of sodium hydroxide in a small test-tube. At the point of contact a blue to red line (dependent upon the amount of dye present) appears. Hemoglobin gives a light yellow line, and bile has been found not to interfere. This test can be carried out on quantities of plasma sufficiently small to permit of blood collection in Wright's blood capsules.

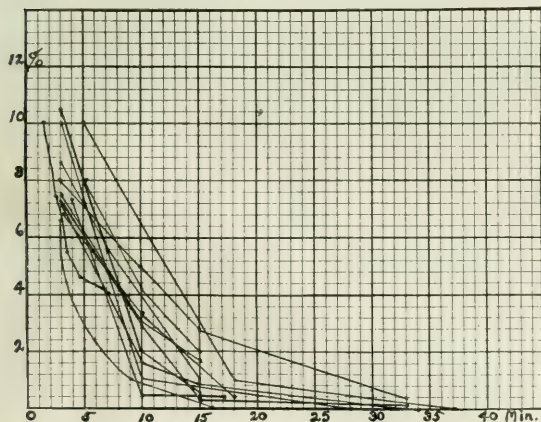


FIG. 3

NORMAL CURVES

With the dosage of 5 mgs. per kilo, there is a most obvious uniformity in the curves obtained. In a series of 12 normal dogs 13 curves varied only 4 per cent at their

most divergent point (Fig. 3). At 3 minutes 6 to 10 per cent concentrations were present, and at 15 minutes determinations ranged from a large trace (3 per cent) to complete disappearance of the dye. The "ring test" often remained positive for 30 to 45 minutes.

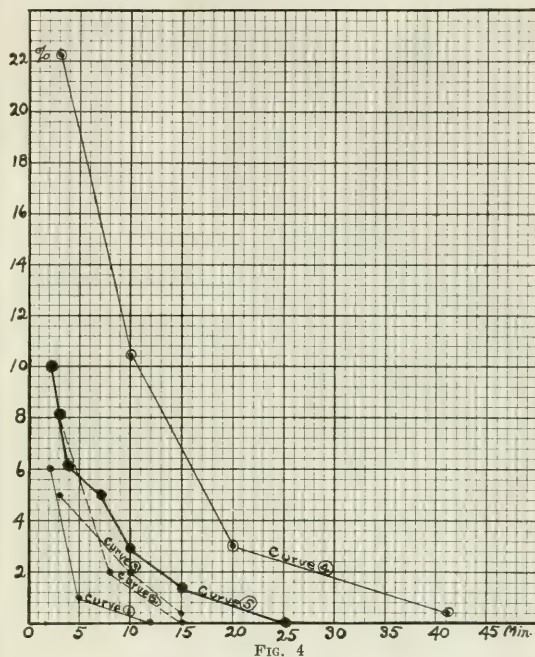


FIG. 4

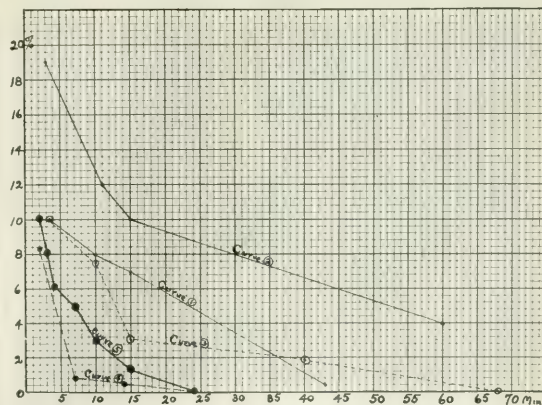


FIG. 5

VARIATION OF DOSAGE

To determine the influence upon the concentration that the dye reaches in the plasma and its rate of disap-

pearance, doses of the phthalein varying from 1.7 mgs. of 10.0 mgs. per kilo have been employed in several dogs (Fig. 4), and in one cat (Fig. 5). It is seen that the curves obtained bear a very definite relation to the amount used. It is also observed that with the usual (5 mgs. per kilo) dose, two cats (Fig. 5) showed somewhat higher curves, and one rabbit a slightly lower curve, than was obtained with the same dose in dogs. These experiments emphasize the importance of adhering to a fixed dosage per body weight, and also suggest that different curves might pertain for different species of animal.

PHOSPHORUS POISONING

Phosphorus, in large doses, causes widespread necrosis and autolysis of the liver parenchyma.^{16, 17, 18} Pathologically, there is a close resemblance to the lesions found in acute yellow atrophy. In the earlier stages the liver is enlarged; the lobules are pale yellow in color, and the liver cells that remain alive are filled with fat. In non-fatal cases the organ soon decreases in size, and in the second week it becomes smaller than normal, microscopically showing connective-tissue proliferation, and often evidence of repair.^{19, 20, 21}

Four dogs were given moderately large doses of phosphorus in oil, intra-muscularly. Prior to the injection they were starved for 24 hours, and following it they were kept upon a carbohydrate-free diet. The following protocol is included:

Dog 18. Male, weight 7.3 kilos. Light ether anesthesia while curves were obtained (See Fig. 6).

June 16: 36.5 mgm. tetrachlorphthalein injected into right external jugular. Curve 1 obtained. After experiment, 12 mgs. phosphorus in oil injected into gluteal muscles.

June 17, 18: Dog does not appear ill. No bile in urine.

June 19: 36.5 mgs. phthalein injected. No cholemia, no bile in urine. Curve 2 obtained.

June 20, 21: Animal is listless, and looks moderately toxic. Urine not dark, but contains bile.

June 22: Above dose of dye injected. Curve 3 determined. Moderate amount of bile in plasma and urine. Slight icteroid tinge to sclerae. Dog appears somewhat ill.

June 23 to 26: Dog brightened up considerably. Jaundice disappearing.

June 26: Normally active and playful. No icterus of sclerae. Urine not dark; trace of bile (Gmelin's test) in plasma and urine. Curve 4 obtained. Animal killed. On autopsy liver edge 2 cm. above costal margin. Color not abnormal. Some increase in connective tissue. No gross evidence of increase in fat. Microscopic examination showed a few scattered areas, chiefly in the centers of the lobules, where the liver cells had been replaced by connective tissue containing many leucocytes. These areas were interpreted as zones of focal necrosis, from which the necrotic cells had been removed, and in which almost complete repair had taken place.

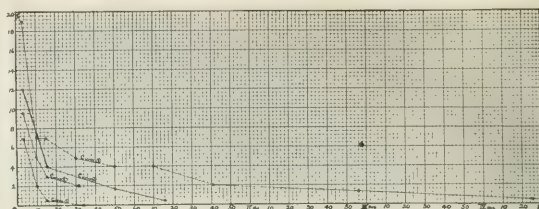


FIG. 6

When ether was employed, the injection of the dye was given within 15 minutes after the anesthetic was begun, so that the effect of ether upon liver function, as shown by Whipple,²² could be neglected. Paraldehyde anesthesia was used during the experimentation upon dog 6. 15 mgs. of phosphorus in oil were given intramuscularly. Four days later the animal was quite ill; considerable bile was present in the plasma and urine; on this day a high curve was obtained (Fig. 7). The animal died while under anesthesia. Autopsy showed the characteristic liver changes of phosphorus poisoning.

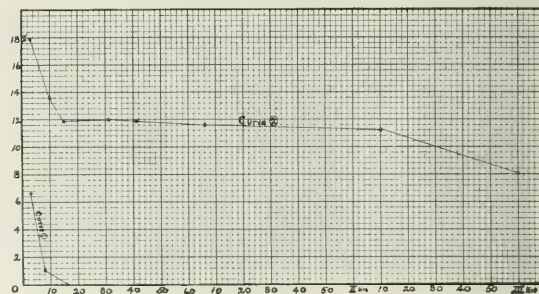


FIG. 7

Dog 14 (Fig. 8) became quite ill by the second day following the injection of 15 mgs. of phosphorus, although little bile was present in either the plasma or urine. A moderately high curve was found at this time; the low readings were rendered only approximately accurate because of hemolysis. The animal died at the end of the experiment.

The normal curve of dog 16 was obscured by lipemia. Forty-eight hours after the injection of 15 mgs. of phosphorus, the phthalein test was again made, and readings fell within the range of early abnormality; the animal did not appear ill. Five days after the injection, icterus was present, and the dog appeared to be in a toxic condition. A curve characteristic of moderately severe liver damage was found (Fig. 9). The animal was killed because of a septic condition of the neck; the liver presented typical gross changes of necrosis and fatty degeneration.

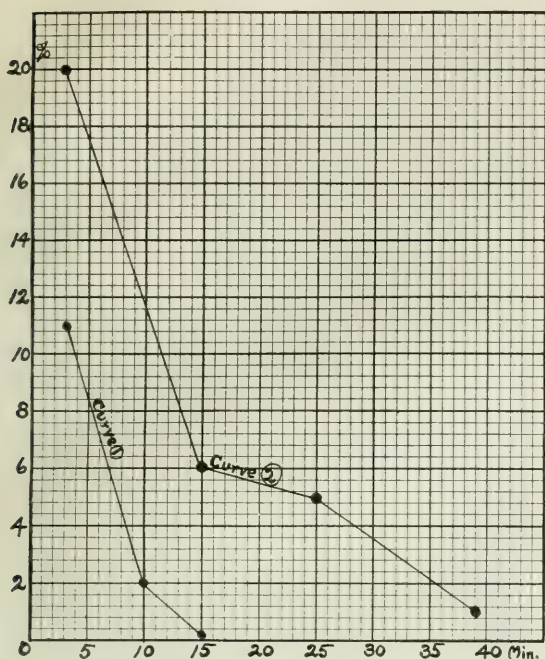


FIG. 8

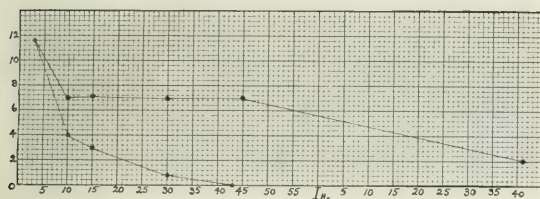


FIG. 9

LIGATION OF THE COMMON DUCT

In acute obstructive jaundice there is dilatation of the biliary passages behind the obstruction; the hepatic cells and cells of Kupfer become deeply pigmented, but there is very slight cell degeneration, except after prolonged obstruction.^{23, 24, 25, 26, 27} The liver cells apparently continue their functions, including the excretion of bile, which is taken up by the blood capillaries and lymphatics.^{28, 29, 23, 25}

It therefore seemed of interest to find out how rapidly the dye would be removed from the blood stream following ligation of the common bile duct. After the normal disappearance time had been determined, the common ducts of five dogs were ligated, and after allowing varying intervals of time to elapse, curves were again obtained. The striking fact was brought out, that in these

animals the tetrachlorophthalein was removed from the blood stream in normal, or almost normal time, except that a trace of dye remained present in the blood as long as these experiments were continued.

TABLE II.

Minutes	2	3	4	5	10	15	30	45	1 Hr.	2 Hrs.	3 Hrs.	4 hrs.
Dog No. 3*												
Day before Ligation	15.			8.	T.	T.	T.					
Dog 1 hour after	13.				T.	T.						
Dog No. 7												
2 days before Ligation	10.				T.		O.					
Dog 2 days after	12.5			3.9.	F.T.	F.T.	F.T.	F.T.				
Dog No. 10												
3 hrs. before Ligation			7.3	F.T.	F.T.							
Dog 2 days after	10.			F.T.	F.T.							
Dog No. 24												
1 hour before Ligation	7.			3.	V.F.T.							
Dog 2 days after	10.			5.	L.T.	L.T.	L.T.	L.T.	L.T.	L.T.	L.T.	L.T.

Per cent of dye in plasma

* The high curves in Dog No. 3 are due to poisoning by chloroform administered a week previously.

FIG. 10

Only one hour had elapsed after ligation in dog 3, when the second test was made; there was no essential difference from the curve obtained previous to ligation (Fig. 10). No bile had appeared in the plasma or urine. Two days after ligation dogs 7, 10 and 24 received their second injections of the dye. The cystic ducts as well as the common ducts were ligated in dogs 10 and 24. All of these animals became icteroid. Curves very slightly higher than the normals for these dogs were reached in the second determinations, but the differences are negligible (Fig. 10).

A curve was obtained in dog 21 three days after ligation of the common duct (Fig. 11). It shows the practically normal rate of disappearance and the persistence of a trace of dye in the plasma is demonstrated, in that, twelve hours after this injection of dye, the "ring test" on the plasma was positive and at this time a large amount of phthalein was present in the urine. Twenty-four hours after the injection, the ring test on the plasma was just barely detectable, and the dye was still present in a catheterized specimen of urine. Following the injection of phthalein in these animals with obstructive jaundice, varying amounts appeared in the urine of all of them except dog 3, who was killed 45 minutes after the injection was made. The appearance of this dye in the urine during experimental obstructive jaundice has been previously studied by Whipple.⁴

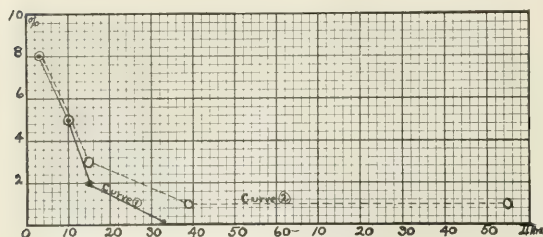


Fig. 11

It is felt that these curves are doubly significant. First, it seems probable that the taking up of the dye by the liver cells, and its excretion in the bile, are independent functions, bearing in mind that chronic obstruction will undoubtedly produce cell damage. The former function seems to be dependent upon the extent and degree of degeneration of the liver cells. The latter function and the time of appearance of the dye in the bile would appear to be influenced greatly by the patency of the biliary passages, and would not necessarily be an index of the condition of the liver cells.

Secondly, while it is felt unwise to draw broad conclusions until the test has been given the trial of time and usage, the writer wishes to point out that, in these experiments, a method has been demonstrated that differentiates the early jaundice due to mechanical obstruction in which cell damage is slight, from the jaundice associated with degeneration of the hepatic epithelium. Experiments are being conducted with phenolsulphonphthalein to determine whether an analogous behavior occurs in renal conditions, in an attempt to develop a test for kidney function upon the same principle.

SUMMARY AND CONCLUSIONS

Phenoltetrachlorophthalein has been intravenously injected and its behavior in the blood stream has been studied in normal animals, in dogs following chloroform poisoning, phosphorus poisoning, and in dogs after ligation of the common and cystic bile-ducts.

The concentrations reached in the plasma of normal dogs, after 5 mgs. per kilo injections, are strikingly constant. Approximately 10 per cent is present in 2 minutes, 8 per cent in 3 minutes, and from a large trace to absence in 15 minutes. The rate of disappearance was found higher in two cats, and more rapid in one rabbit. Variation of the dose has a direct influence upon the curve obtained.

There is a marked increase in these concentrations, with a greatly prolonged rate of disappearance, after injury to the liver parenchyma; the extent to which this occurs depends upon the degree of damage to the liver cells, as evidenced by the condition of the animals, and by autopsy findings. Curves approaching normal are obtained if repair has taken place.

Curves have been obtained in jaundice due to mechanical obstruction that differ widely from those found in the jaundice associated with extensive degeneration of the hepatic cells.

A simplified method is herein presented for quantitative determination of the amount of dye in the plasma. A practical test for its qualitative detection is also described.

A clinical study is in progress, and a series of cases will be reported in the near future.

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LEGENDS

Fig. 1.—Dog No. 5. Chloroform Poisoning. May 5, normal curve (1). May 7, two hours and fifteen minutes chloroform anæsthesia. May 8, curve (2), eighteen hours later. Dog not very ill; chloroform again given for one hour. May 9, curve (3), second day. May 12, curve (4), fifth day. May 18, curve (5), eleventh day.

Fig. 2.—Table I. Preparation of series of standards to which specimens of undiluted plasma are compared directly. 10 mgs. of phenoltetrachlorphthalein to 100 c.c. of water used as 100 per cent solution of dye.

Fig. 3.—Curves of disappearance of phthalein from blood stream. 13 curves on normal dogs; 5 mgs. per kilo dosage.

Fig. 4.—Variation of the dosage of dye in normal dogs. Curve (1), Dog No. 11: 1.7 mgs. of tetrachlorphthalein per kilo. Curve (2) and curve (3), Dog No. 12 and Dog No. 13: 2.5 mgs. per kilo. Curve (4), Dog No. 15: 10 mgs. per kilo. Curve (5), composite curve constructed from Fig. 3, 5 mgs. per kilo.

Fig. 5.—Curves on normal cats and rabbit. Curve (1), Cat No. 2, 5 mgs. per kilo. Curve (2), Cat No. 2, 10 mgs. per kilo. Curve (3), Cat No. 3, 5 mgs. per kilo. Curve (4),

Rabbit No. 1, 5 mgs. per kilo. Curve No. 5, composite curve from Fig. 3.

Fig. 6.—Dog No. 18. Phosphorus poisoning with convalescence. Curve (1), before intramuscular injection of 12 mgs. of phosphorus. Curve (2), three days after injection. No icterus; dog not ill. Curve (3), six days after injection. Bile in urine and plasma; moderately toxic. Curve (4), eleven days after injection. Dog appears normal; small trace of bile in urine.

Fig. 7.—Dog No. 6. 15 mgs. of phosphorus. Curve (1), normal. Curve (2), four days after injection. Animal quite ill; moderate jaundice.

Fig. 8.—Dog No. 14. 15 mgs. of phosphorus. Curve (1), before injection. Curve (2), two days later. Dog very toxic.

Fig. 9.—Dog No. 16. 15 mgs. of phosphorus. Curve (1), (above), two days after phosphorus injection. Curve (2), (below), five days after injection: dog moderately ill, slight jaundice.

Fig. 10.—Table II. Curves before and after ligation of common bile-duct.

Fig. 11.—Ligation of common bile-duct. Dog No. 21. Curve (1), before ligation. Curve (2), three days after ligation; deep jaundice.

A STUDY OF TRICHOMONAS HOMINIS, ITS CULTIVATION, ITS INOCULATION INTO ANIMALS AND ITS STAINING REACTION TO VITAL DYES

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In a previous paper the author¹ has shown that a pure line of *Trichomonas hominis*, i.e., a race started from a single individual, can be isolated, and that this pure line can be kept in culture, the medium best suited for this purpose being Locke-egg. In this medium the organism has to be transferred every two or three days, since the cultures reach their greatest development on the second or third day and rarely live more than five or six days.

In the fall of 1921 a very simple method was devised, by which *Trichomonas hominis* can be easily kept alive in test-tube cultures for thirty-five days and in some cases for over sixty-six days, without being transferred. This greatly facilitates the work of keeping cultures in stock for experimental work.

The stock, from which the pure line used for these experiments was isolated, has been cultured for eight months in this laboratory. The pure line was started when the stock was two and one half months old, and has been kept under constant observation for the past five and a half months.

Sodium chloride serum water medium. The method used is the following: To a flask containing 100 c.c. of 0.85 per cent sodium chloride solution, which has been sterilized in the autoclave, add 10 to 15 c.c. of sterile sheep serum water. For these animals sheep serum is better than pig serum, though the latter can be used.

The serum water is prepared by diluting one part of sheep serum with three parts of distilled water, and is sterilized in the Arnold sterilizer, being heated at 100° C. for one hour on three successive days. This solution will then have a pH of 7 to 7.4. After thoroughly mixing, pour 15 c.c. of this solution into sterile test tubes and inoculate with *Trichomonas hominis* by means of a capillary pipette, the transferred material being deposited at the bottom of the test-tube. The surface of the medium is then covered with sterile paraffin oil to prevent evaporation and the tubes are incubated at 36° C.

The serum in the medium very soon coagulates, owing to the action of the bacteria which are growing in the culture with the *Trichomonas*. This coagulated material settles to the bottom. It is here that the organisms can be found in largest numbers.

Trichomonas hominis does not divide rapidly in these deep cultures covered with paraffin oil. In counting the organisms in one field (oc. 10, obj. 16 mm.) one rarely finds more than eight or ten individuals and usually there are not more than three or four in a field. At this slow rate of division the waste products do not accumulate rapidly and the culture lives for a long time.

When large numbers of *Trichomonas* are needed for experiments, 10 c.c. of the sodium chloride serum water medium are put into sterile tubes and inoculated with a

few drops of a culture containing *Trichomonas*. This is incubated at 36° C. In twenty-four hours there will be a rich growth of *Trichomonas* and in forty-eight hours the culture will be swarming with them. Over a hundred have been counted in one field (oc. 10, obj. 16 mm.).

In making counts to determine the relative number of individuals present in a culture, it was necessary to determine whether the organisms are evenly distributed throughout the medium. Samples of the culture were taken from the surface by means of a platinum loop and the number of organisms in one field (oc. 10, obj. 16 mm.) were counted. Specimens of the same culture from the bottom of the tube were taken by means of a capillary pipette, and were studied with the same magnification. The count for the organisms on the surface in five fields was: 15, 14, 12, 17, 13; and for those from the bottom of the same tube, 66, 94, 92, 85, 104. Many such counts were made, but the organisms in the bottom of the tube always greatly outnumbered those on the surface. This would seem to suggest that they are dependent on the bacteria which are collected in great masses in the coagulated serum. Further evidence for this was seen when the surface of the tubes occasionally became covered with a growth of bacteria. The *Trichomonas* would then often be found swarming on the surface. As many as 280 and 300 individuals have been counted in fields taken from such a surface.

Sodium chloride serum water was made up in flasks, and, with phenol red as indicator, its reaction was adjusted by the colorimetric method to pH 8.4, 8.2, 8, 7.8, 7.6, 7.4, 7.2, 7, 6.8. The scale for this indicator does not run below 6.8, but two other flasks were made up which had a pH of about 6.6 and 6. This was only approximately estimated by the color, but the fluids were known to be decidedly acid. The adjustment was made by adding hydrochloric acid and sodium hydroxide.

From these flasks 10 c.c. were put into sterile tubes by means of sterile pipettes and each tube inoculated with 5 c.c. of a culture of a pure line of *Trichomonas hominis* which had been grown in a flask to insure a sufficient quantity for inoculating all the tubes from the same

culture. Examinations were made after forty-eight and ninety-six hours. The tubes were first shaken to insure an even distribution of the organisms. Then two loops full of the culture were transferred to a slide and the organisms in three fields counted (oc. 10, obj. 16 mm.).

From the accompanying table it will be seen that *Trichomonas* grows in an acid as well as in an alkaline medium. The most favorable range of reaction for rapid multiplication is pH 7.2 to pH 8.4, with pH 8 as an optimal point. If it is desired to keep the cultures for a longer time, pH 7.2 and pH 7.4 are better. Here the rate of division is slower and consequently the waste products, which are very poisonous to the organisms, do not accumulate rapidly so that the organisms can live longer.

Animal Experimentation. Several authors have reported inoculating animals with *Trichomonas*. Escomel² said he infected the rabbit, guinea-pig, cat and dog with *Trichomonas*. Lynch³ claimed to have infected a white rabbit with *Trichomonas* from a culture tube by injecting them three inches up into the rectum. Three days later he said the rabbit was passing soft brown stools containing many *Trichomonas*, which had not been previously present. Boyd⁴ said he infected a rat by feeding it milk which contained a portion of a culture of *Trichomonas* and what he called *Trichomonas* cysts. The *Trichomonas* was not plentiful and he said: "It remains to be ascertained whether infection by feeding results from the flagellates or from the cysts. By analogy the latter would seem probable and my single feeding experiment, taking into consideration the paucity of flagellates present, would tend to support this view." At the present time it is generally recognized that cysts of *Trichomonas* have not been seen and it is very doubtful whether they are ever formed. With this in mind the results of Boyd seem questionable. Recently Pringault⁵ fed *Trichomonas* to a cat, guinea-pig, rabbit, and white rat and injected them into the rectum of a kitten. All his attempts at inoculation gave negative results.

With these discordant results in mind it was decided to try infecting cats and rabbits, paying especial attention to preliminary fecal examinations, as it has been shown that many intestinal protozoa appear in the stools at intervals several days apart, and, unless the infected animals are watched for a period of at least four or five days, the parasites which they carry cannot be accurately determined.

Accordingly three young cats were isolated and daily fecal examinations made for seven days. None of them was infected with flagellates. They were then fed, by means of a pipette, two-day-old cultures full of *Trichomonas hominis*. Each cat received from 8 to 9 c.c. of the culture. For six succeeding days the stools were examined microscopically, but *Trichomonas* did not appear and the stools were normal.

pH.	Number of tubes inoculated	Number of fields examined	Average number organisms in each field of 48-hrs. culture	Average number organisms in each field of 96 hrs. culture
6. (?)	5	15	5.8	0
6.6 (?)	5	15	9.1	0
6.8	4	12	20.4	4.5
7.	4	12	20.	4.3
7.2	2	6	46.	8.2
7.4	2	6	54.5	10.
7.6	2	6	38.8	5.7
7.8	2	6	51.3	0
8.	2	6	87.	1.2
8.2	2	6	20.1	4.7
8.4	2	6	44.1	.2

Four kittens, which were three weeks old, were next used. The stools were examined for seven days and found not to contain *Trichomonas*, though all were infected with *Giardia*, *spirochaetes* and *Belascaris*. On the seventh day they were fed 10 c.c. of a culture of *Trichomonas hominis* containing 20-24 organisms in each field (oc. 10, obj. 16 mm.). Daily fecal examinations were made for five days but *Trichomonas* did not appear. Then 10 c.c. of a three-day-old culture were injected into the rectum of each kitten. The rectal tube was passed two and a half inches up into the large intestine. After this, fecal examinations were made for six days with negative results. The kittens were again inoculated *per rectum* with 10 c.c. of a culture containing 25 to 35 *Trichomonas* per field. The succeeding eleven fecal examinations were negative. A third attempt was made to infect them *per rectum*, but fecal examinations covering five weeks did not reveal any *Trichomonas*.

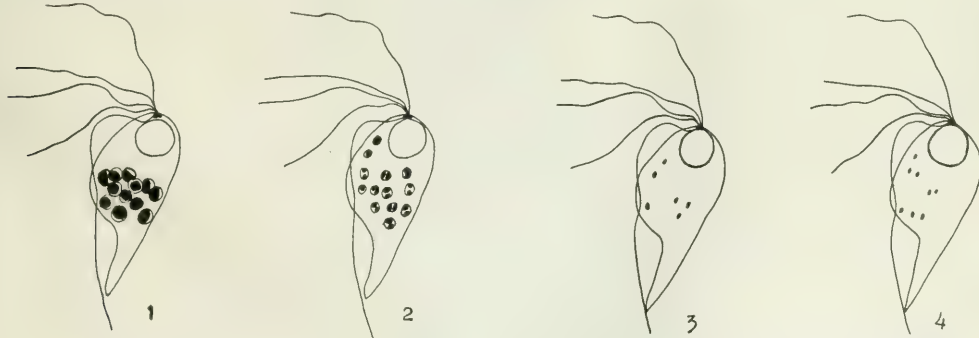
Since both Escomel² and Lynch³ have reported successful inoculation of rabbits with *Trichomonas*, these animals were next tried. Two rabbits were examined daily for nine days and found not to be infected with *Trichomonas*, though one of them was infected with a flagellate closely resembling *Trichomonas* which appeared at irregular intervals, often three days apart. The flagellate did not have an axostyle nor an undulating membrane. It resembled somewhat a *Prowazekia*. It was very plastic. Both rabbits were given *per rectum* 13 c.c. of a rich culture of *Trichomonas*. On eight succeeding days fecal examinations did not show *Trichomonas*, though the other flagellate still appeared at irregular intervals. A second inoculation of 17 c.c. of the culture was given *per rectum*, but the eight succeeding examinations were negative.

An attempt was made to inoculate two white rabbits, since Lynch³ especially designated the use of this kind of rabbit. On these animals preliminary stool examinations were made for four days, which were negative. They were then inoculated with 25 and 30 c.c., respectively, of a rich culture of *Trichomonas*. The eight succeeding fecal examinations were all negative.

From the above experiments the conclusion has been drawn that cats, kittens, and rabbits cannot become infected with *Trichomonas hominis*. Organisms closely resembling *Trichomonas* were found in the stools of rabbits before and after inoculation. The question is opened whether they have not possibly misled some of the former investigators who claim to have inoculated these laboratory animals with *Trichomonas*.

Vital Stains. These rich cultures of *Trichomonas hominis* offered a good opportunity for studying the effect of vital stains on an intestinal protozoan parasite. Neutral red, methylene blue, brilliant cresyl blue and pyrrol blue were all used in varying dilutions in hanging drops of the culture. The coverslips were sealed with vaseline. Neutral red stained granules in vacuoles which were usually situated in the posterior two thirds of the body (Fig. 1). A few neutral red vacuoles were occasionally seen around the nucleus in the anterior part of the body (Fig. 2). The granules in the vacuoles varied in size and in number, as has been shown to be the case with *Amoeba*.⁶ Brilliant cresyl blue stained the granules a purplish blue and the vacuole pink. Methylene blue stained the granules blue. Pyrrol blue did not stain any part of the *Trichomonas*.

Mitochondria. With janus green the mitochondria stained a blue green. They were short thick rods arranged in groups and singly (Figs. 3, 4). They were not numerous.



The outlines of Figs. 1, 2, 3, and 4 were made with a camera lucida from a prepared slide ($\times 3000$ diameters). The neutral red granules in Figs. 1 and 2 and the mitochondria in Figs. 3 and 4 were put in by free hand, as the animals were moving too rapidly to draw with the camera lucida.

Fig. 1 shows large neutral red vacuoles containing single neutral red granules.

Fig. 2 shows the neutral red vacuoles containing neutral red granules of different sizes. The vacuoles are distributed throughout the body.

Figs. 3 and 4 show the short rod-shaped mitochondria which are scattered about in the body of the *Trichomonas*.

SUMMARY

1. Sodium chloride serum water has proved an excellent medium for the cultivation of *Trichomonas hominis*. When the tubes are covered with paraffin oil the organisms live from 35 to 66 days without being transferred.

2. *Trichomonas* multiplies most rapidly on sodium chloride serum water medium with a pH 8. It lives longest on the same medium with a pH 7.2-7.4.

3. Attempts were made to infect cats, kittens and rabbits with *Trichomonas hominis* but all attempts failed.

4. *Trichomonas hominis* contains granules which take

the vital stains, neutral red, methylene blue and brilliant cresyl blue.

5. Mitochondria in the shape of short blunt rods are present and stain with janus green.

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ENTERO-URETHRAL FUSION IN A FETUS, SIMULATING FETAL ASCITES

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About 80 cases of congenital fusion of the intestinal and urinary tracts have been described in the literature. These can be divided into a variety of types. In some, the intestine communicated with the bladder, in others, with the urethra. In most instances the junction was in the midline; in many, however, the colon emptied into one side of an asymmetrical bladder. The bladder was dilated in relatively few of the cases. With rare exceptions, the abnormality has occurred in males, but a closely related malformation in the female, in which the rectum opens into the vagina, is quite common. A survey of the literature dealing with the subject revealed no case as young as the one here described and none in which the prostatic urethra participated in the formation of the bladder cavity. Most of the reported cases were those of infants born alive, on whom the diagnosis was made because of the passage of meconium through the urethra in the absence of an anal orifice. Surgeons have made use of the nature of the mixture of urine and meconium to locate the site of the fusion. If the fluid passed at the beginning of micturition was meconium and later clear urine was passed, it was assumed that the colon emptied into the urethra; whereas, if the fluid was a mixture of urine and meconium, then the point of union was assumed to be in the bladder. In either case, the communication was usually so small as to preclude the possibility of satisfactory defecation and to make early operation imperative.

The fetus described in this paper (male, 85 mm. crown-rump length, Carnegie Collection No. 2547) was presented to the laboratory by Dr. N. W. Ingalls, of Cleveland, as a case of fetal ascites. Examination showed a bizarre creature with an enormously distended abdomen which made the extremities appear small by contrast (Fig. 1). The external genitalia were so under-developed as to leave the question of sex in some doubt until after

examination of the gonads. The anal orifice was missing and there was no clear indication of the normal site of the anus. As fetal ascites is characterized by a disproportionately large abdomen, in the absence of peripheral edema this diagnosis was accepted as correct. The existence of an imperforate anus excited no suspicion of the true condition, since one abnormality is frequently associated with others. Furthermore, a dead-born infant with an imperforate anus alone does not, as a rule, show abdominal distention. At autopsy, however, it was found that the abdominal enlargement was due to an abnormally dilated bladder (Fig. 2).

The thoracic viscera showed no gross anomaly. The liver and small intestine were removed and found to be normal. The kidneys were normal in size and position and sections of them showed no dilatation or cyst formation. The sex glands, which lay just below the pelvic brim, proved to be testicles, thus establishing the sex of the fetus. The ureters were not dilated as far as they could be followed behind the large bladder. A section of the bladder wall showed the musculature to be made up of non-striated fibers of the usual bladder type.

Transverse sections 100 μ in thickness were made from a level just below the lower poles of the kidneys downward through the genitalia. These sections were stained with hematoxylin and eosin. The outstanding malformation shown by a study of these sections is an abnormal relation between the descending colon and the urinary tract. The colon opens into the prostatic urethra, which in turn is dilated to such an extent that it has become anatomically a part of the bladder. The bladder, as can be seen from figure 2, is enormously dilated. The wall of the fundus shows little if any hypertrophy, while in the region of the trigone the mucous layers appear to be somewhat thickened and infiltrated by cells with deep-staining nuclei. The posterior wall of the bladder is



FIG. I



FIG. II

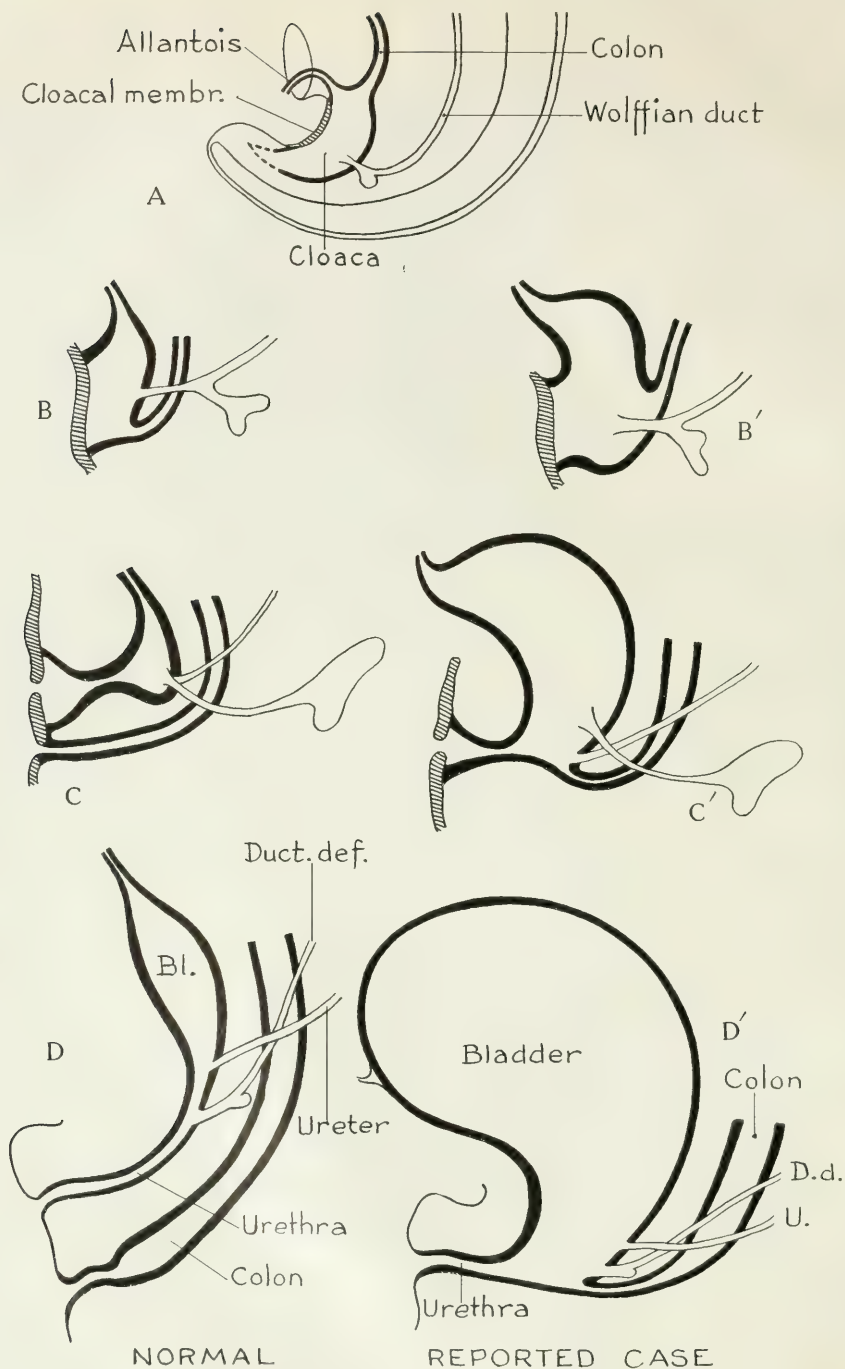


FIG. 3

folded sharply backward upon itself so as to form a deep longitudinal sulcus, at the base of which the urethra leaves the bladder. Running upward along the posterior wall of the sulcus, from the opening of the urethra, is a band of mucosa strikingly different from that covering the rest of the bladder and resembling closely that of the urethra, with which it is continuous. From this point the urethra can be followed to the external meatus on the rudimentary penis. Its lumen, although filled with cell debris in places, is clearly patent. From the point where it leaves the bladder to the meatus it is not dilated. Normally, the prostatic urethra at this age is relatively wide, with its lumen continuous with the bladder cavity. In this specimen it has widened still further until it has become anatomically a part of the bladder. This appears to account for the band of urethral mucosa found on the bladder wall and is confirmed by the relation which the deferential ducts and seminal vesicles bear to this band of mucosa. The ducts at this age normally join the prostatic urethra a little below the bladder, as has been shown by Johnson.¹ In this specimen they are lost in the bladder wall just lateral to the band of urethral mucosa mentioned above. In other words, that part of the bladder cavity from which the mouth of the urethra opens is not, from a developmental standpoint, bladder at all, but is in reality a dilated prostatic urethra. The membranous portion of the urethra, instead of its prostatic portion, constitutes the exit from the bladder cavity.

The ureters follow a normal course and join the bladder in the usual locations. The right ureter is slightly dilated as it passes through the bladder wall and at its point of entry the bladder is folded upon itself so as to form a pocket.

The descending colon in its upper part appears normal in all respects. As it approaches the posterior wall of the bladder, however, it narrows rapidly until the lumen is barely distinguishable. The muscle layers retain their normal thickness throughout, but the mucosa becomes thin and atrophic as the lumen narrows. This abnormal colon penetrates the posterior wall of the prostatic urethra just above the exit of the membranous urethra from the bladder. No further traces of the intestinal canal are found at lower levels. These relations are shown schematically in figure 3, D'.

DISCUSSION

An interpretation of this condition requires answers to several questions, on some of which our knowledge is inadequate.

What happened to interfere with the usual separation of the urinary and alimentary tracts, and when? The normal transition from the early cloacal phase to complete separation of these tracts is shown in figure 3-A.B.C.D. It will be seen that a wedge of tissue pushes down-

ward, forcing rectum and urethra apart. In our specimen something happened, in the interval between the stages represented by diagrams A and C, to check the descent of this wedge, leaving the colon to empty into the prostatic urethra. In figure 3, D' represents the condition found in this embryo, while B' and C' represent an attempt to bridge the gap between D' and normal A. As B shows the relations in an embryo of about five weeks, normal development must have been interfered with at about this time, probably in the first half of the second month.

Why did the bladder dilate? The simple answer that it was distended by increased internal pressure is not a satisfactory one. In the first place, there is no evidence that at this age there is any secretion from kidneys or intestine that might dilate the bladder. In the second place, the ureters, colon, and urethra are all patent and open freely into the vesico-urethral cavity. Any increase in the hydrostatic pressure in the bladder, therefore, would have caused fluids to be forced either into the colon and ureters, thus dilating them, or through the urethra, thus relieving the pressure within the bladder. The ureters, however, are not dilated and the colon is actually narrowed. Hence it is clear that the bladder was not distended from increased internal pressure. The only other explanation that presents itself is that the bladder dilated intrinsically and was filled with fluid only in order to avoid a vacuum.

Anders² explains dilatation and asymmetry of the bladder in these cases as being due to an overgrowth of that organ, similar in nature to neoplastic growth. There is, moreover, a certain amount of experimental support for the belief that trophic changes in the embryo may cause dilatation in a hollow viscus. Streeter³ found in the tadpole that marked dilatation had developed in ear vesicles that had been transplanted to abnormal sites.

Finally, what caused the death of the fetus? That the abnormality itself was the cause is highly improbable, as there is nothing about it incompatible with fetal or even postnatal life. Two other possibilities present themselves. First, that the ovum itself was originally defective and that this defect caused both the abnormal development and the death of the fetus. Second, that the original germplasm was healthy, but that some process, perhaps a toxemia on the part of the mother, so profoundly influenced the embryo as to cause the abnormalities and later its death. Between these possibilities there is no ground on which to base a decision.

I wish to express my sincere thanks to Dr. G. L. Streeter for his many helpful suggestions in the pursuance of this study and for his kindness in extending to me the facilities of the Carnegie Laboratory of Embryology. I also wish to thank Mr. Charles Miller for the preparation of the serial sections.

SUMMARY

A fetus is described, the external appearance of which provoked a diagnosis of fetal ascites.

The abdominal enlargement is due to an enormously dilated bladder. The prostatic urethra is so dilated as to form part of the bladder cavity.

The descending colon empties into the prostatic urethra.

The dilatation of the bladder is thought to be due to overgrowth of bladder tissue rather than to distention.

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DESCRIPTION OF FIGURES

Fig. 1.—Embryo erroneously diagnosed fetal ascites, showing large abdomen. Carnegie Collection No. 2547.

Fig. 2.—Same embryo with abdominal wall folded back, and liver and intestines removed to show large bladder.

Fig. 3.—A. Early relations of colon and urinary tract drawn from model of normal fetus of 5.5 mm. greatest length. Carnegie Collection No. 1380. B. Relations in normal fetus of 7 mm. From Felix,⁴ Fig. 577A. C. Relations in normal fetus of 11 mm. *Ibid.* Fig. 604A. D. Relations in normal fetus of 85 mm. From dissection. B' and C' represent stages in the reported case corresponding to B and C, inferred from Normal A, and relations as found in D'. D'. Relations in reported case as established by serial sections.

REPORT OF A CASE OF TUBAL PREGNANCY PROBABLY CAUSED BY A PAROVARIAN CYST

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Although it has long been known that tubal pregnancies can be caused by any factor that interferes with the course of the fertilized ovum as it travels inward along the Fallopian tube, this case presents several rather unusual features which seem to justify its publication.

The brief summary of the case is as follows:—F. B. Gyn. No. 154077. Age 26—white—female. Admitted: February 24, 1922.

Family and general past history unimportant. Menstrual history normal until the onset of present illness. Marital history: Married one year, one child born September 1921, that is, five months before the present admission to the hospital, when the patient was still nursing it.

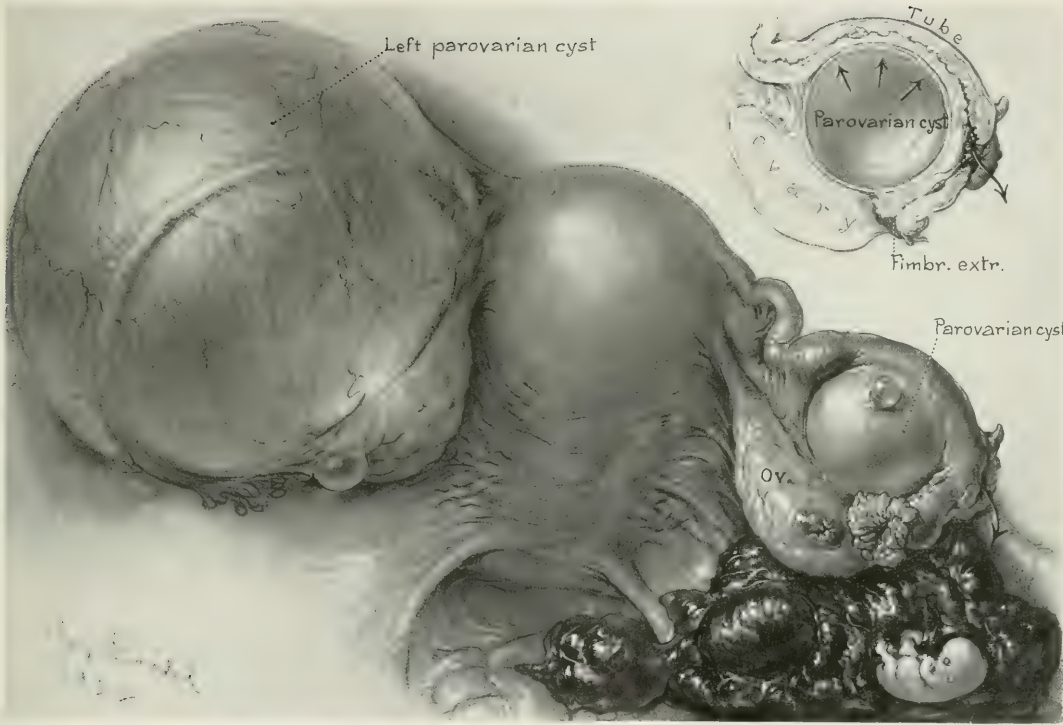
Present Illness.—Three months after the birth of her normal child, that is, two months before admission to the hospital the menstrual period returned. Five weeks later a second period of normal duration occurred. Three weeks later, that is, sixteen days before admission to the hospital, she began to bleed again, although the menstrual period was not due until a week later. The bleeding was not of greater quantity than usually occurred with the periods, but instead of stopping at the end of four or five days continued for sixteen days until the patient was admitted to the hospital. Two days before admission to the hospital the patient felt "generally run down" and went to her doctor who prescribed calomel and salts. The next day she was taken suddenly with a drawing pain over the whole lower abdomen which was so severe that it compelled her to go to bed. The pain was especially bad at stool. She says she felt as if she were going to die.

The pains became more severe during that day and the following night. The next day the attending physician noticed the facial pallor, localized the abdominal tenderness in the lower abdomen and sent the patient into the hospital with a diagnosis of ruptured tubal pregnancy.

Admission Note.—The patient is a well developed white woman in extreme prostration, but mentally oriented, complaining of intense dryness of the mouth, weakness and abdominal tenderness. Mucous membranes quite pale. Temperature 98.4°; pulse 140, weak and irregular. Respirations 28, white blood count 15,000. Red blood count 2,160,000. Hemoglobin 33%. Blood pressure, 96/74. General physical examination essentially negative. The breasts are swollen and tender and contain milk. General abdominal tenderness is present. Shifting dullness is demonstrable in the flanks. Pelvic examination: Outlet marital, mucous membranes very pale. A slight amount of bloody vaginal discharge. Cervix soft. Filling the whole cul-de-sac there is a soft mass which to the examiner has the characteristic consistency of blood-clots.

Pre-Operative Diagnosis.—Ruptured extrauterine pregnancy.

When the patient was first seen, it was at once evident that she could not in her condition stand an operation and, unless something were done very soon, she would die. The husband's blood fortunately matching with hers, a transfusion of 600 c.c. of citrated blood was given in the operating room. Although the patient had a slight chill and a rise of temperature after it, her general con-



dition improved at once and the blood pressure rose from 96 to 120. One hour after completion of the transfusion the operation was begun. After division of the fascia and separation of the muscles, the blood-clots filling the abdominal cavity could be seen shining through the peritoneum, and when the peritoneum was opened, there was an outpouring of a large quantity of blood. The entire pelvis was filled with blood, and blood was seen pouring from a rupture which had occurred in the right Fallopian tube at a distance of about 2 cm. from its fimbriated end. There was a small parovarian cyst present which had kinked the tube just proximal to the point where the tubal pregnancy had occurred and which was in all probability the causal factor of its development. On the left side there was also a parovarian cyst, but this was much larger, measuring 12 cm. in diameter. The tube on the left side was drawn over the cyst. The ovaries on both sides were perfectly normal. I removed the Fallopian tube on the right with the extrauterine pregnancy, both parovarian cysts and as much of the blood-clots from the abdominal cavity as I could without prolonging the operation. At the termination of the operation the pulse was 160 but of good quality.

Convalescence was quite satisfactory and the patient was discharged in good condition twenty-two days after her operation.

Pathological Report (Dr. Warrinner).—Gyn. Path. No. 27577.

The right tube measures 8 by 2.5 cm. in its distal third. The tube is ruptured 1.5 cm. from its fimbriated end. Just under the point of rupture is a parovarian cyst. This cyst is thin-walled and filled with clear fluid. In the center of one of the large blood-clots removed at operation the fetus and membranes are found. The fetus measures 5 cm. in length.

In addition to being one of the many cases we now see of tubal rupture with complete collapse of the patient, in which a transfusion improves the condition sufficiently to allow the performance of a successful operation, this case presents the two following interesting factors:

(1) This patient developed a tubal pregnancy only five months after the termination of a normal pregnancy and while she was still nursing her baby. (2) It is seldom that one can demonstrate so clearly a mechanical factor causing the arrest of the fertilized ovum in the tube as is shown in this instance.

For the excellent drawing of the conditions found at operation I am greatly indebted to Mr. Max Broedel.

LEGEND

Posterior view of pelvic conditions as found at operation. On the right is shown the parovarian cyst which had kinked the tube at a point immediately proximal to the site of the rupture. On the left the larger parovarian cyst is shown with the elongated and thinned out Fallopian tube extending over its top. The small drawing in the upper right hand quadrant indicates the mechanism by which the right tube has been compressed by the parovarian cyst.

THE ISOLATION OF NUCLEIC ACID FROM TISSUES

By WALTER JONES AND CASPAR FOLKOFF

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Johns Hopkins Medical School)

The well known chemical distinction between animal and plant nucleic acid suggests an inquiry into the chemical nature of bacterial nucleic acid to learn whether in this respect bacteria are to be regarded as animals or plants. Attempts to decide this question failed for want of a method of isolating nucleic acid that is applicable to the material which constitutes the bodies of bacteria, until recently, when one of us (Folkoff), in collaboration with Schaffer and Bayne-Jones,* prepared a considerable amount of bacterial nucleic acid and found the substance somewhat unique. The method of isolation employed by these experimenters had been previously elaborated by us in connection with yeast and is here described for purposes of reference.

Five liters of cold fresh brewer's yeast diluted with eight liters of cold tap-water are treated with 2½ liters of 20% sodium hydroxide in small successive portions, and the mixture is stirred continually for ten minutes,

care being taken that the temperature of the material does not rise. At the end of this time most of the alkali has been neutralized with hydrochloric acid. The solution is finally made faintly but distinctly acid to litmus with acetic acid and after standing over night in a cold place the muddy opalescent dark brown fluid is decanted from the sharply settled yeast detritus and treated with hydrochloric acid and alcohol in the usual way for the precipitation of the nucleic acid, which is washed by decantation with alcohol of increasing strength and finally filtered tightly on a Buchner funnel.

The crude nucleic acid thus obtained is ground in a mortar with five or six parts of water containing an excess of ammonia, and after the solution of the nucleic acid is complete, an equal volume of alcohol is added. The excess of ammonia is then carefully neutralized with acetic acid. This is the crux of the method. *The excess of ammonia must not be neutralized until after the alcohol has been added*, and the end-point of the neutralization is determined not with an indicator but by the

* Schaffer, Folkoff and Bayne-Jones: Johns Hopkins Hospital Bull., 1922, XXXIII, 151.

appearance of a bulky dark brown precipitate leaving an easily filterable interstitial liquid that is as transparent and nearly as colorless as distilled water.

The clear fluid is filtered off, treated with an equal volume of alcohol and the precipitated snow-white flakes are washed and dried with alcohol. As this process of purification does not involve an appreciable loss of material, it may be repeated as often as is suggested by the appearance of the final material, and can be applied to the purification of any of the commercial preparations of yeast nucleic acid.

The final product (30 grams of which are obtained from five liters of yeast) consists of a colorless biuret-free

amorphous powder which dissolves easily and completely in water to form a colorless solution. It yields decomposition products which bear the quantitative relation to one another that is required for the ammonium acetate addition product of yeast nucleic acid and contains exactly half of its phosphorus in easily split form, showing that its purine and pyrimidine groups are present in equivalent amounts.

As the method described cannot be applied to the isolation of nucleic acid from animal glands, its successful execution in a particular case would suggest that the questionable material under examination is of plant origin.

A CASE OF CONGENITAL OSTEOSCLEROSIS

By RALPH K. GHORMLEY, M.D.

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(From the Orthopedic Service of the Surgical Department
of the Johns Hopkins Hospital)

The following case is presented because of its unique characteristics and in the hope that, if others are recognized, they will be brought to the knowledge of the profession.

CASE HISTORY

J. C. (Surg. No. 55907), male, white, *æt.* 8, was first seen in the Orthopedic Dispensary of the Johns Hopkins Hospital on February 1, 1922.

Complaint: "Pain in left hip and limp."

F. H. The father and mother are living and well. There is one half brother and one half sister by a former husband of the mother. Since her marriage to the father of the patient there has been one child born prematurely, who lived two months; then there were five miscarriages all before the end of the fourth month. The patient is the last child born. There is no history of either syphilis or gonorrhoea in the father or mother.

P. H. Premature birth at eight months. Weighed four pounds. Breast fed. The child developed quite normally, however, and aside from varicella, at the age of three years, there have been no acute illnesses. Tonsillectomy and adenoidectomy were performed one year ago. The mother states that two years previous to the present illness the child had some "growing pains" in the back for a short time.

P. I. During the first week in December, 1921, the boy complained of pain in the left hip which was more marked at night and toward the close of the day. This pain would also be noticed after resting from his play. The parents noticed a slight limp. There was no history of any injury. There was never any swelling and no night cries. The condition has not progressed to any appreciable extent.

Physical examination. Blood pressure, 100/65; pulse, 80; respiration, 18; temperature, 99.2; Height, 4 ft., 3 in.; weight, 58 lbs.

The patient is a fairly well developed and nourished boy; he is bright and active. There is no suggestion of any mental retardation (Fig. 1).

Head: Negative.

Neck: Negative, thyroid not palpable.

Chest: Heart and lungs clear, no retro-manubrial dullness, no rachitic rosary.

Abdomen: The abdomen is rather prominent. The walls are everywhere soft. There is no tenderness anywhere and no masses are felt. None of the organs are palpable.

Glands: There is some enlargement of the posterior cervical lymph glands which are discrete and not tender. The axillaries and epitrochlears are not felt. The inguinals are moderately enlarged, discrete and not tender.

Skeletal System. Spine: The spine is quite straight, although there is slight deviation toward the right to compensate for the slight tilting of the pelvis toward the left. The movements of the spine are free in every direction and there is no tenderness anywhere. *Upper extremities:* The muscular development is good. There is no bony tenderness or thickening. The joints are all normal. *Lower extremities:* The child walks with a moderate limp toward the left. The left foot is held rotated outward when standing. There is slight atrophy of the thigh and calf on the left. When he lies down, there is about half a centimeter of apparent shortening of the left leg. Voluntary as well as passive movements of the left hip reveal slightly less abduction than on the right. There is a slight decrease in the hyperextension of the hip. There is no muscle spasm and no tenderness. The measurements are:

	Right	Left
Apparent shortening.....		0.5 cm.
Ant. sup. spine—Int. malleolus.....	64 cm.	63.5
Great trochanter—Ext. malleolus.....	61.5	61.5
Base of Bryant's triangle.....	4.5	3.75
Circumference of thigh.....	33.5	32.5
Circumference of calf.....	24.5	24.
Blood. Hemoglobin 70% (Sahli)		
R. B. C. 4,600,000		
W. B. C. 7,050		
Differential count:		
P. M. N.	56.3%	
P. M. E.33	
P. M. B.33	
L. Lymph.	2.33	
S. Lymph.	36.3	
L. Mono.66	
Trans.	3.66	

There is slight anisocytosis and poikilocytosis. Hemolysis resistance tests are normal.

Platelet count, 128,000.

Reticulated R. B. C., 0.9%.

Blood Wassermann, negative; ice-box preparation, negative.

Blood Chemistry:

N. P. N., 30 mgm. per 100 c.c.

CO₂, 47.1 vols %

Sugar, .119

Phosphorus, 4.8 mgm. per 100 c.c.

Calcium, 10.2 mgm. per 100 c.c.

The blood culture showed no growth.

Urine: Sp. Gr. 1018; acid; albumin and sugar tests negative; microscopic examination negative.

Basal Metabolism, 11.1% above the average for the age and sex.

During the course in the hospital, while under observation, there was an almost daily afternoon rise in temperature to about 100° F. Nothing was found to account for this. Operation was refused by the parents, so no exploration could be carried out. A plaster of Paris spica was applied with the leg as fully abducted as possible without anesthesia. This was worn for about three weeks after which time a radiograph showed little change in the hip. The boy, however, had no further pain and the limp seems to be less.

DISCUSSION

On seeing the patient in the dispensary our impression was that we were dealing with a beginning epiphyseal separation of the head of the femur on the left. Radiographs of the pelvis taken at that time showed a curious dense appearance of the bones. This was thought at first to be an artefact, but carefully repeated pictures showed the same condition (Fig. 2). Radiographs of all of the bones were then made and showed much the same condition throughout the skeleton. Beside the curious density of the flat bones and the rather hazy outline along all of the bones, the vertebral bodies showed a marked density at either pole (Fig. 3). There is thickening of the cortex of the ribs (Fig. 4), the marrow cavity in places being apparently obliterated. There is definite

thickening of the cortex of all the long bones, more marked in the femora and humeri (Figs. 5 and 6), and the cortical thickening seems to be greater in the proximal portion of these bones than in the distal portion. The skull (Fig. 7) is thicker than normal. In the femoral necks, and especially in the left, there seems to be a breaking down of the bone just below the epiphysis so that the epiphysis is slipped inward and downward (Fig. 2).

Radiographs of the mother and father were then made, which showed the mother's bones to be normal but that practically the same condition existed in the father as in the child (Figs. 8 and 9). The father's history was then reviewed and it was found that both of his parents had lived to be eighty and that he has seven brothers, all of whom are living and in good health. It is hoped that we may be able later to get radiographs of some of these brothers. The father states that when he was a boy he had "growing pains" in his legs, but had no treatment and entirely overcame them after adolescence. The blood Wassermann of the father, as well as of the mother, was negative. Smears of the father's blood were normal. No dietary discrepancy in either father or son could be discovered.

A search through the literature has failed to locate any cases similar to this in the living person. There are, however, several cases described at autopsy as osteosclerosis, some as congenital and others as associated with disturbance of the blood-forming organs.

Frangenheim¹ has collected three cases from other writers of autopsy reports of congenital osteosclerosis. Two of these were in infants with a normal blood picture but a third, a case reported by Goodall² was in a child who died at the age of ten weeks of what is described as acute myelocythemia, of three weeks' duration, with bleeding from the mucous membranes, enlargement of the liver and spleen and a blood picture of myeloid leukemia. Beside the changes in the organs the bones showed osteosclerosis of the ribs, sternum, femora, humeri and tibiae. Another of these cases was originally reported by Schmidt of a child who died after thirty hours with enlarged thymus, normal blood smears and an apparently normal bone-marrow. The bones showed marked thickening of the cortex of the long bones, skull and sternum and a marked density in the vertebral bodies. The other case cited by Frangenheim was originally reported by Assmann. A child died, when one day old, showing beside enlargement of the thymus and thyroid a normal blood picture and normal bone-marrow. There was an associated diffuse sclerosis of the long bones as well as of the skull and vertebrae. In all of these cases the cortical thickening was mostly central with partial obliteration of the marrow cavities, there being some cartilaginous rests and the marrow in places being encased in thick-walled bony spaces.

Assmann³ has collected in addition four cases in adults who died at ages ranging from 17 to 68 years.

Two died of leukemias and the others, it was thought, of primary anemias. All of these cases showed similar bone changes at autopsy.

Neuwerck and Moritz¹ report one case with similar bone changes but draw no conclusions except to refute the statement of Schlazzenhofer² that the osteosclerotic process may be due to arsenic or phosphorus used in the medication. Neither of these drugs had been used in their case.

Schwarz⁶ reports a case of leukemia with osteosclerosis in a woman, 47 years old, who died after a six months' illness, the autopsy showing, beside enlargement of the liver and spleen with findings characteristic of leukemia, a thickening of the cortex especially of the femora and humeri. The ribs, vertebrae and skull also showed sclerosis. In the long bones of this case it is noted that the proximal portion of the cortex was more markedly thickened than the distal portion. The marrow cavity is described as grayish red to dark brown in color with bony trabeculae scattered through the marrow.

Heuck⁷ in autopsies on two cases of leukemia in men, 24 and 25 years of age, found marked thickening of the diaphyses of the femora and humeri, as well as of the vertebrae and skull. He holds that the osteosclerosis is of long standing and that the blood changes are due to the changes wrought in the marrow by the osteosclerotic process, or that the occurrence of the two is a coincidence.

The above case reports are all pathological without any radiographs. However, the anatomical descriptions are so nearly what one would expect to find at autopsy

in the case here reported that we feel we are dealing with the same condition. The majority of the authors take the view that the sclerosis is secondary to an irritation which goes with the morbid process in the blood. Heuck, however, takes an opposite view, as above stated. In our case we seem to be dealing with a definitely inherited condition not related, so far as we know, to any metabolic disturbance which we can demonstrate. There is possibly some increase in the blood phosphorus, the figures given being above the average normal for the age and sex. There is, however, no evidence of either the father or son having been on a high phosphorus diet. It would seem that the condition itself is not incompatible with life and that the secondary blood diseases may come as a coincidence or as a failure of the decreased blood-forming mechanism to respond to the excessive demands placed upon it.

We are greatly indebted to the various laboratories, for aid in the investigations in this case.

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BOYLSTON MEDICAL PRIZES

These prizes, which are open to public competition, are offered for the best dissertation on questions in medical science proposed by the Boylston Medical Committee.

At the annual meeting held in Boston in 1920 a prize of three hundred dollars was awarded to an essay entitled "Acute Inflammation of the Nose, Pharynx and Tonsils" by Mr. Stuart Mudd of St. Louis.

For 1922 there is offered a prize of five hundred dollars and the Boylston Prize Medal, for the best dissertation on the results of original research in medicine, the subject to be chosen by the writer. The Boylston Prize Medal will be added to the money prize only in case the winning essay shows special originality in the investigations detailed.

Dissertations entered for this prize must be in the hands of the Secretary, REID HUNT, M.D., Harvard Medical School, Boston, Mass., on or before February 1, 1923.

In awarding these prizes, preference will be given to dissertations which exhibit original work, but if no dissertation is considered worthy of a prize, the award may be withheld.

Each dissertation must bear, in place of the author's name, some sentence or device, and must be accompanied by a sealed packet, bearing the same sentence or device, and containing the author's name and residence within.

Any claim by which the authorship of a dissertation is made known to the Committee will debar such dissertation from competition.

Dissertations must be printed or typewritten, and their pages must be bound in book form.

All unsuccessful dissertations are deposited with the Secretary, from whom they may be obtained, with the sealed packet unopened, if called for within one year after they have been received.

By an order adopted in 1826, the Secretary was directed to publish annually the following votes:

1. That the Board does not consider itself as approving the doctrines contained in any of the dissertations to which premiums may be adjudged.
2. That, in case of publication of a successful dissertation, the author be considered as bound to print the above vote in connection therewith.

The address of the Secretary of the Boylston Medical Committee is REID HUNT, M.D., Harvard Medical School, Boston, Mass.



Fig. 1. —Showing distortion and deformity, the result of coxa vara.

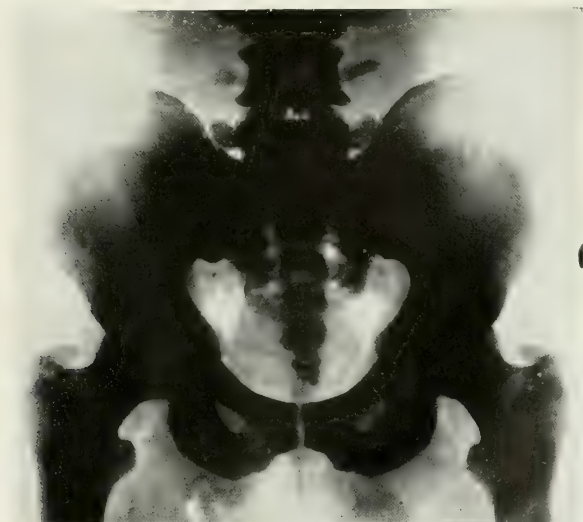


Fig. 2.—Osteosclerosis of all the pelvic bones with partial subluxation of the epiphyses of the femora and some new bone formation around the epiphyseal lines.

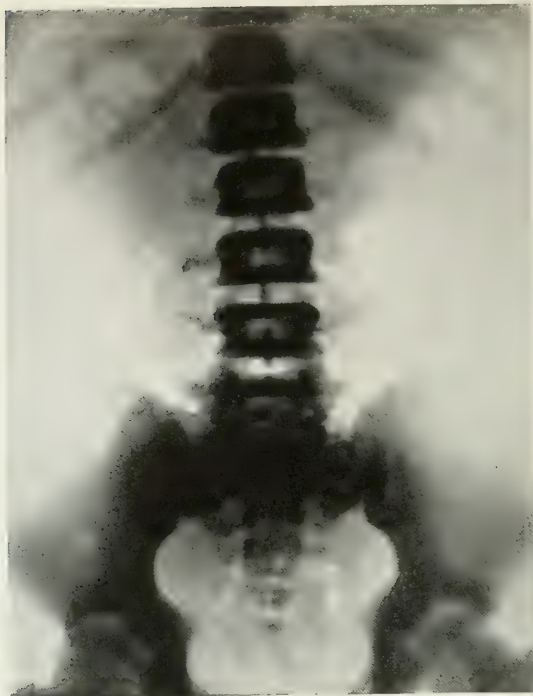


Fig. 3.—Showing marked osteosclerosis of the poles of the vertebræ.

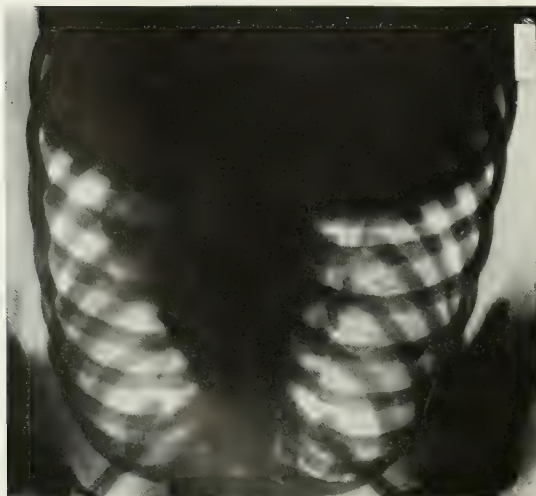


Fig. 4.—Showing thickening of the cortex of the ribs.



Fig. 5.—Showing a marked thickening of the cortex.



Fig. 5a.—Showing a marked thickening of the cortex.



Fig. 6.—Showing a marked thickening of the cortex.

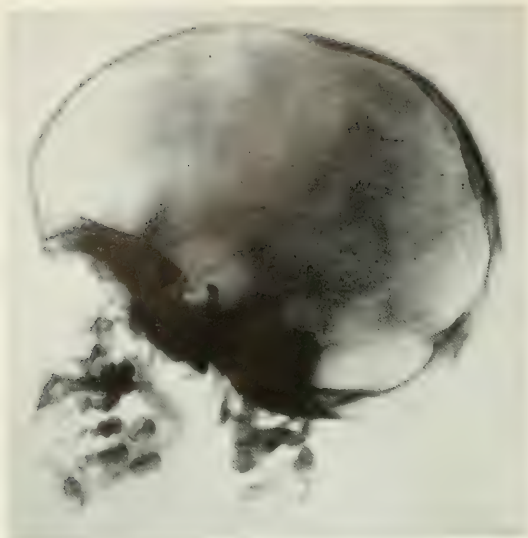


Fig. 7. -Marked osteosclerosis of the bones at the base of the skull.



Fig. 8.—Osteosclerosis of the pelvic bones of patient's father. These show no subluxations of the epiphyses.

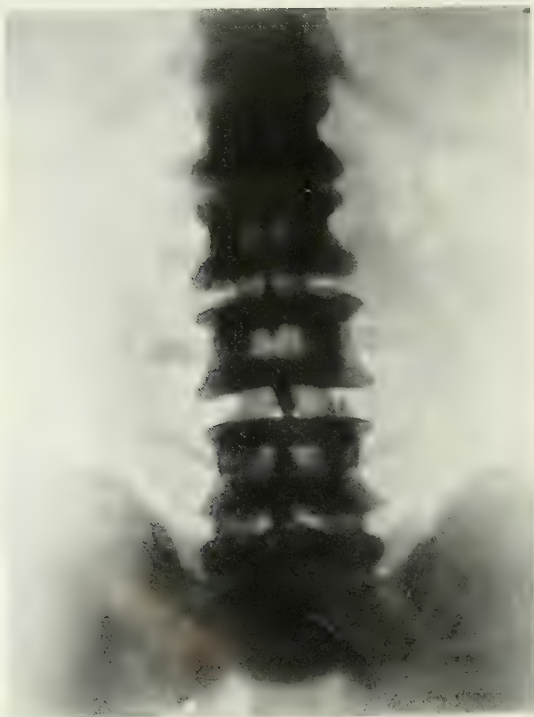


Fig. 9. -Spine of patient's father, showing the same osteosclerotic condition of the poles of the vertebræ.

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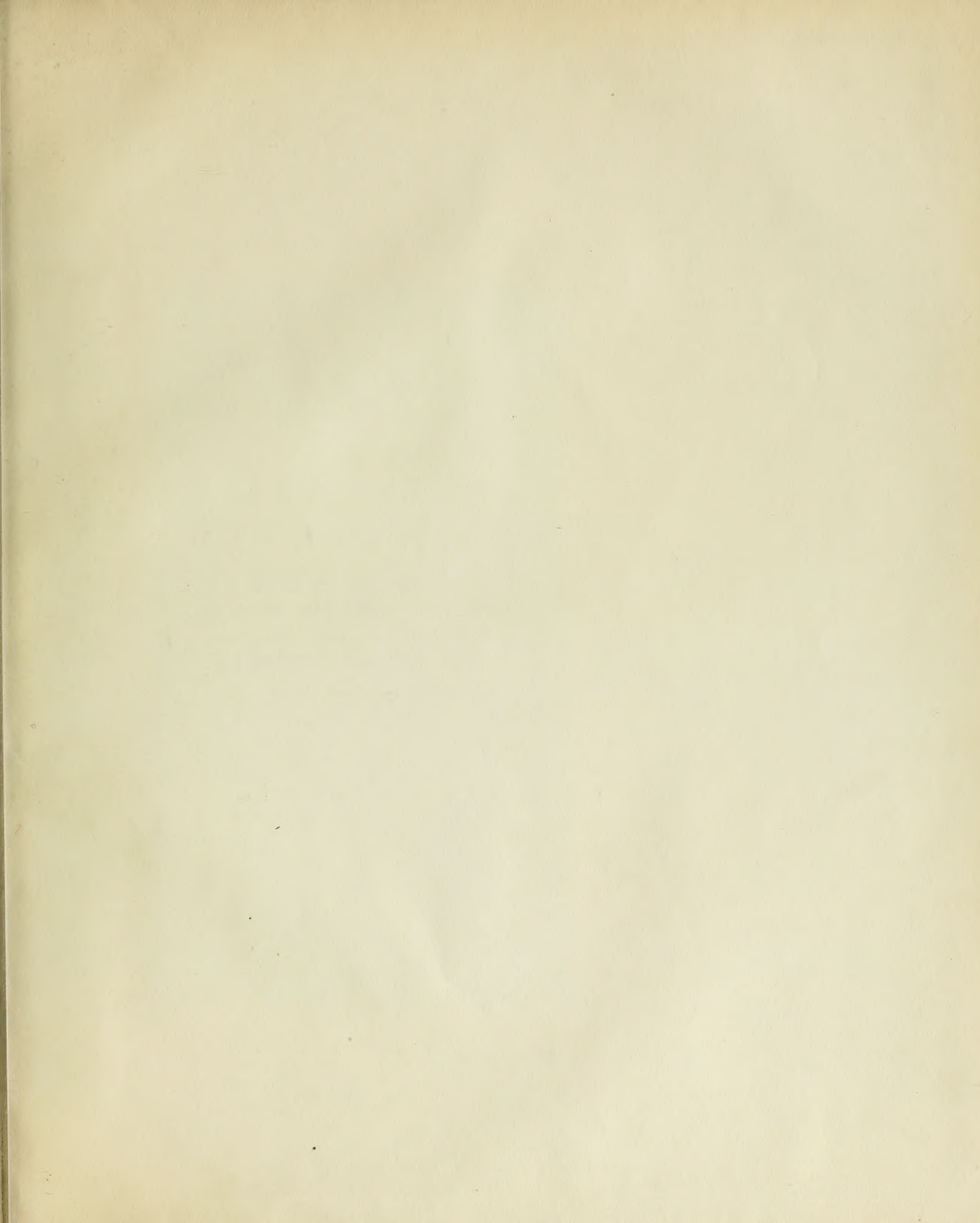
Attention is called to two mistakes in the article by Dr. J. Whitridge Williams entitled "The Influence of the Treatment of Syphilitic Pregnant Women upon the Incidence of Congenital Syphilis" which appeared in the November Bulletin.

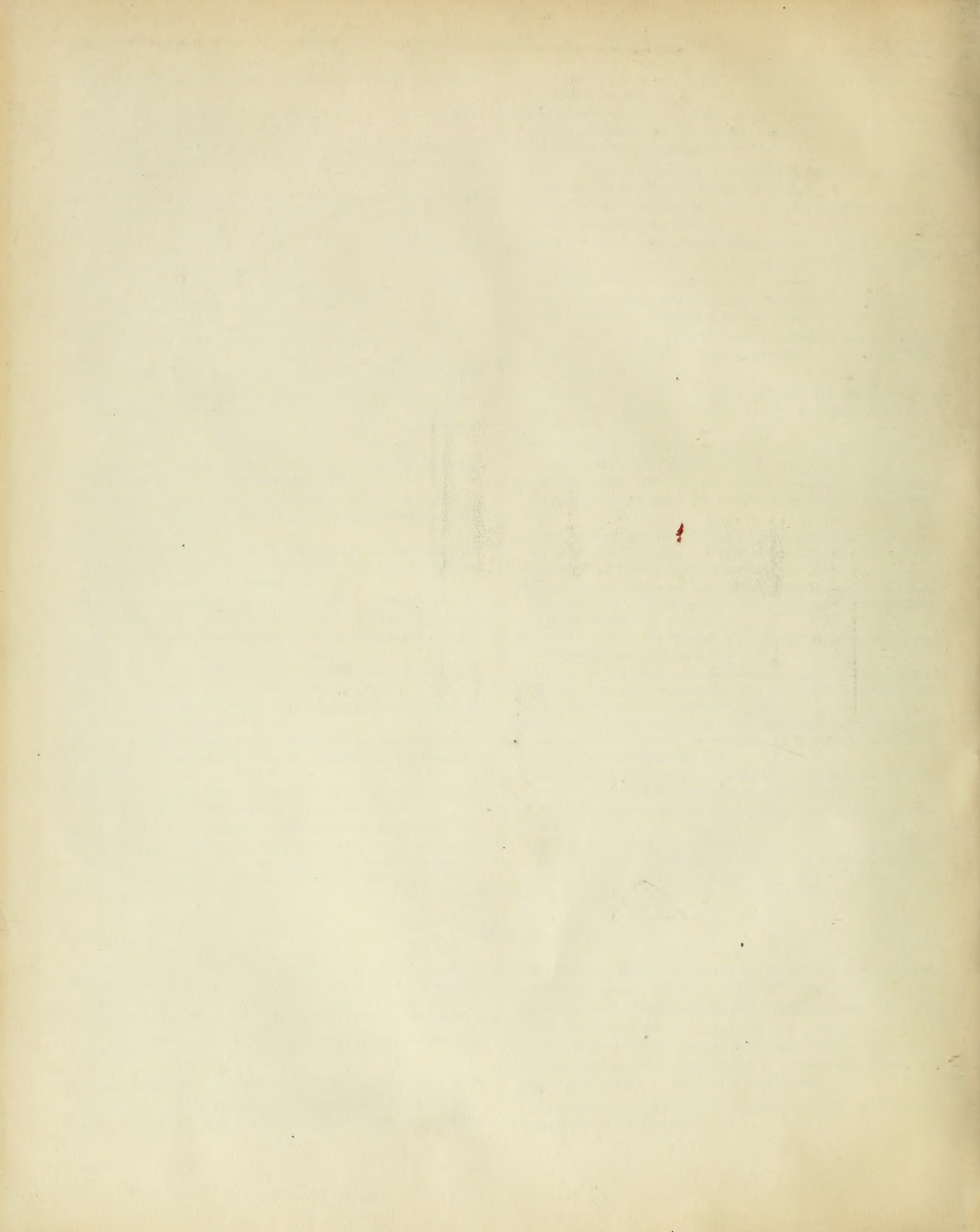
On page 386, second column, the last paragraph should read as follows:

In conclusion, I am aware that the present paper is based upon too small a material to possess any statistical value, but at the same time the results which we have

obtained are highly suggestive and are interesting from two main points of view. First, because our study is based upon the observations made upon a series of women with whose past history we have long been familiar, and secondly, because the results obtained are extraordinarily stimulating from the standpoint of treatment and of the great hope which it offers for the future.

On page 385, Table II, the total for "Deaths after Treatment" should be 9 instead of 3.





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